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YELLOW FEVER INSTITUTE, BULLETIN No. 14.

Treasury Department, U. S. Public Health and Marine-Hospital Service.

WALTER WYMAN, *Surgeon-General.*

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REPORT

OF

WORKING PARTY NO. 2,

YELLOW FEVER INSTITUTE.

EXPERIMENTAL STUDIES IN YELLOW FEVER AND MALARIA AT VERA CRUZ, MEXICO.

BY

M. J. ROSENAU, Passed Assistant Surgeon.
HERMAN B. PARKER, Passed Assistant Surgeon.
EDWARD FRANCIS, Assistant Surgeon.
GEORGE E. BEYER, Acting Assistant Surgeon.

MAY, 1904.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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PREFACE AND ACKNOWLEDGMENTS.

Working Party No. 2, Yellow Fever Institute, consisting of Passed Asst. Surg. Herman B. Parker, Asst. Surg. Edward Francis and Acting Asst. Surg. George E. Beyer, was detailed by the Surgeon-General U. S. Public Health and Marine-Hospital Service, with the approval of the Secretary of the Treasury, to Vera Cruz, Mexico, April 27, 1903, for the purpose of making further studies upon the cause and methods of transmission of yellow fever.

Doctor Parker returned to Washington upon official business June 7, 1903, and on September 13, 1903, returned to Vera Cruz with Passed Asst. Surg. M. J. Rosenau, Director of the Hygienic Laboratory, who had in the meantime been appointed chairman of the commission, with instructions to repeat that part of the work of Working Party No. 1 relating to the *Myxococcidium stegomyiæ*.

Professor Beyer left for New Orleans on October 4 to resume his duties at the Tulane University of Louisiana.

The remaining three members of the working party continued work at Vera Cruz until November 28, 1903, when, on account of the subsidence of the yellow fever epidemic and scarcity of material, they returned to Washington.

The commission made a brief report to the Surgeon-General, signed by all of its members and published in the Public Health Reports for January 15, 1904, as follows:

Findings of Working Party No. 1, Yellow Fever Institute, not all corroborated by Working Party No. 2.

WASHINGTON, *December 18, 1903.*

SURGEON-GENERAL.

SIR: We have the honor to report that, as a result of our studies at Vera Cruz, Mexico, this summer, we have not been able to corroborate all the findings of Working Party No. 1, Yellow Fever Institute, Public Health and Marine-Hospital Service, having found phases of the organism described by them as *Myxococcidium stegomyiæ* in normal mosquitoes.

Respectfully,

M. J. ROSENAU,
Chairman Working Party No. 2.
H. B. PARKER,
EDWARD FRANCIS,
GEO. E. BEYER.

The commission was now, January 18, 1904, dissolved, and its members permitted to publish individually any further matter bearing on the summer's work.

This bulletin, therefore, has been prepared by two members of the commission, Doctors Rosenau and Francis, who have continued certain phases of the work, but who here wish to make full acknowledg-

ment of the services rendered by their colleagues. Especial mention should be made of the fact that the mosquitoes which fed on yellow-fever cases and subsequently were used to produce the initial case of experimental yellow fever (Marcos Cruz) were handled by Professor Beyer.

Credit is also due to Professor Beyer for the scheme of experimentation which was partly carried out. This plan was published by him in full in the New Orleans Medical and Surgical Journal for May, 1904, entitled "The mouth parts and salivary glands, normal and otherwise, of the yellow-fever mosquito." Professor Beyer was a member of Working Party No. 2 from May 5, 1903, to January 18, 1904, and was in charge of the laboratory at Vera Cruz from June 8 to September 17, 1903, during the absence of Doctor Parker.

Asst. Surg. Joseph Goldberger was associated with us throughout the entire summer, having been detailed to Vera Cruz to supervise the sanitation of vessels leaving for the United States. He helped us find suitable cases of yellow fever and malaria in the hospitals from which he infected a large collection of mosquitoes, and he also made many of the observations which we have embodied under "Miscellaneous observations on mosquitoes."

The plans of the commission were laid before Governor Dehesa, of the State of Vera Cruz, who was always most zealous in furthering the scientific investigation of yellow fever, and offered us many facilities.

To Mr. Alexander M. Gaw, of Jalapa, Mexico, we desire to express our particular appreciation of many thoughtful kindnesses and material assistance.

To Dr. Eduardo Licéaga, president of the superior board of health of Mexico, and to his representatives in Vera Cruz, Doctors del Rio, Iglesias, and Garcia, we wish to express our thanks for their interest in the work and for many courtesies, thoughtful kindnesses, and material assistance.

The United States consul, Mr. W. W. Canada, and Acting Asst. Surg. S. H. Hodgson, U. S. Public Health and Marine-Hospital Service, were always ready to assist us in every way possible.

Finally, we wish to express our appreciation to the Surgeon-General of the Public Health and Marine-Hospital Service for his continued interest and support which made the work possible.

M. J. Rosenau,
Passed Assistant Surgeon, Chairman.
Herman B. Parker,
Passed Assistant Surgeon.
Edward Francis,
Assistant Surgeon.
Geo. E. Beyer,
Acting Assistant Surgeon.

YELLOW FEVER INSTITUTE.

Treasury Department, Bureau of Public Health and Marine-Hospital Service.

WALTER WYMAN, Surgeon-General.

BULLETIN No. 14.

Section B.—ETIOLOGY. P. A. Surg. M. J. ROSENAU, Chairman of Section.

EXPERIMENTAL STUDIES IN YELLOW FEVER AND MALARIA.

By M. J. ROSENAU, *Passed Assistant Surgeon,*
HERMAN B. PARKER, *Passed Assistant Surgeon,*
EDWARD FRANCIS, *Assistant Surgeon,*
GEORGE E. BEYER, *Acting Assistant Surgeon.*

THE CAUSE OF YELLOW FEVER.

The cause of yellow fever is not known, but we have to consider the *Myxococcidium stegomyiæ* of Parker, Beyer, and Pothier. These authors described in some detail the life cycle of a supposed animal parasite in infected mosquitoes closely resembling coccidia.

It was our first duty to investigate the merits of this announcement.

We therefore first sectioned about one hundred normal mosquitoes, *Stegomyia* and *Culex*, both male and female. A study of these slides soon convinced us that bodies resembling *Myxococcidium stegomyiae* may be found in normal mosquitoes and that for the most part these bodies were yeast cells in various stages of reproduction. Carroll had called our attention to this in a conversation and subsequently discussed it in an article published in the Journal of the American Medical Association for November 28, 1903.

Since then the French commission, working at Rio de Janeiro,[a] has come to the same conclusion.

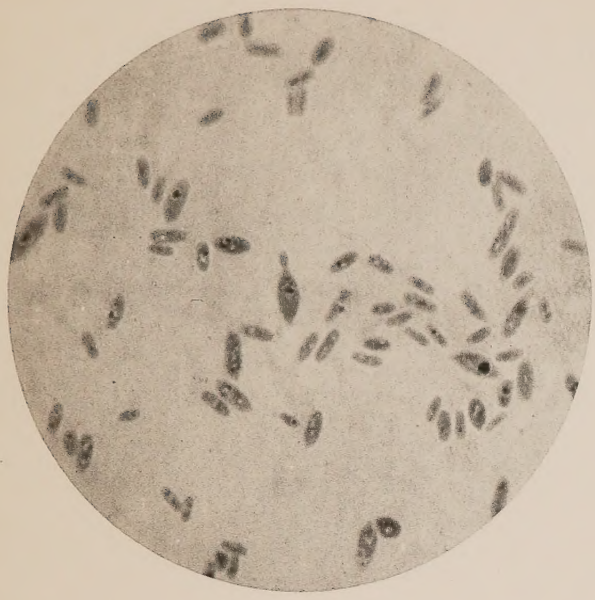
[a] Marchoux, Salimbeni, and Simond: La fièvre jaune; rapport de la mission française. Ann. de Inst. Pasteur, tome XVII, November, 1903.

EXPLANATION OF PLATE 1.

Wild yeasts in pure culture isolated from banana at Vera Cruz, Mexico, in the summer of 1903. Stained with hematoxylin and eosin.

All the specimens show deeply stained granules.

1. Shows the lemon-shaped budding forms of *Saccharomyces apiculatus*.
2 and 4. Other budding forms.
3. Well marked granules.
5. Ovoid forms with granules.
6. Filamentous yeast.



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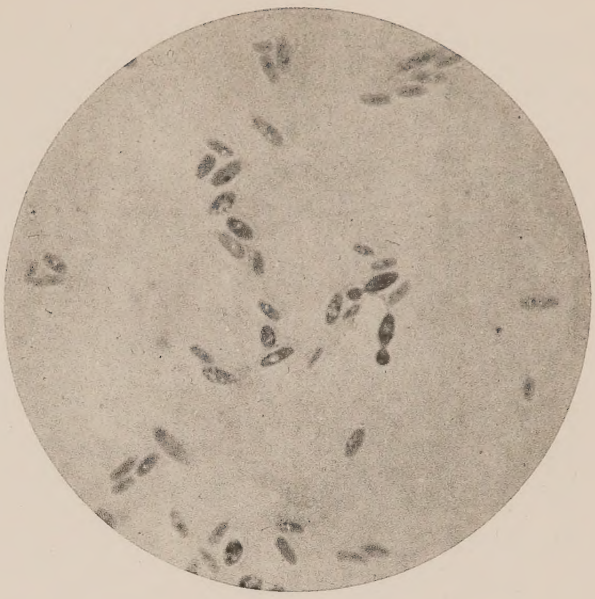

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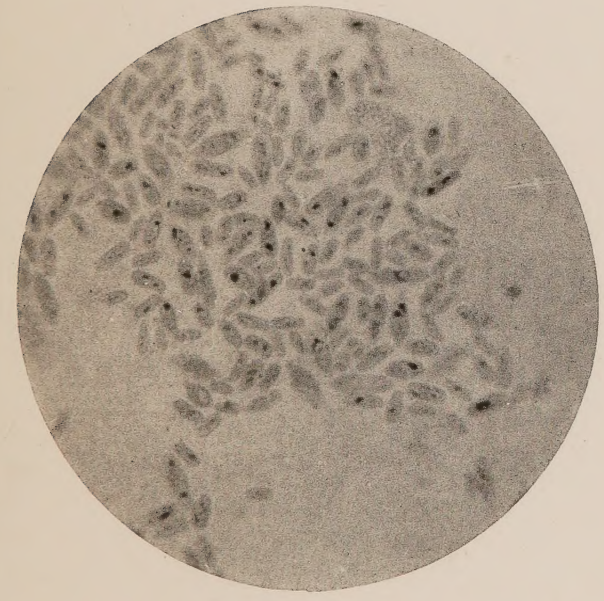

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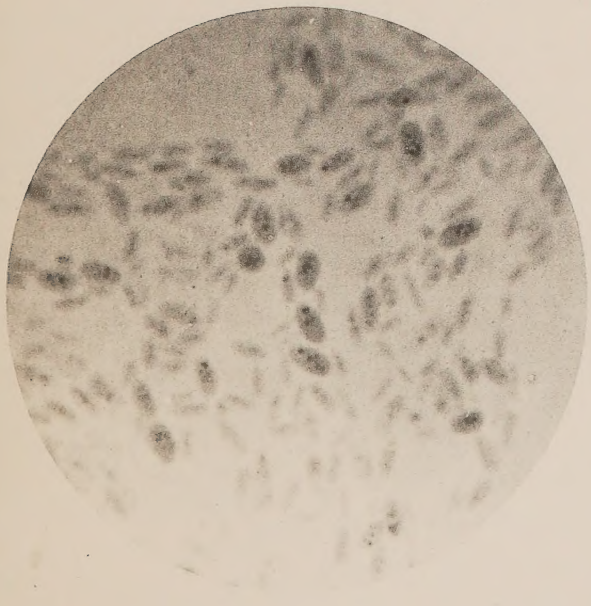

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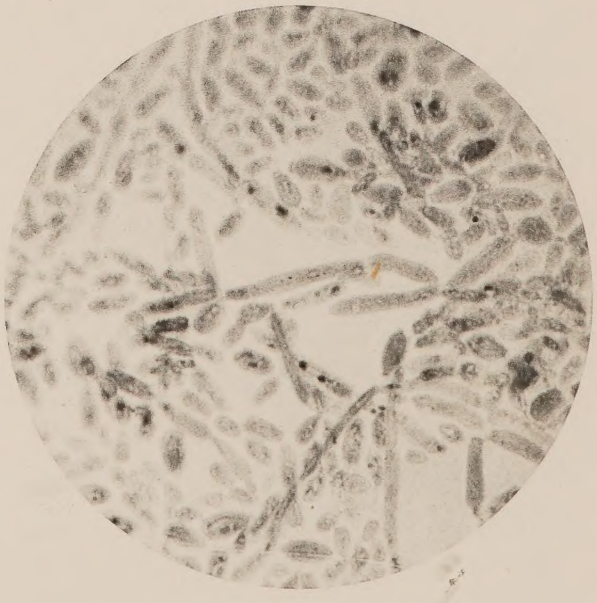

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WILD YEAST IN PURE CULTURE ISOLATED FROM BANANA AT VERA CRUZ, MEXICO, IN THE SUMMER OF 1903. STAINED WITH HEMATOXYLIN AND EOSIN. ALL THE SPECIMENS SHOW DEEPLY STAINED GRANULES.

Schaudinn[a] considers yeasts as normal commensals of all mosquitoes and believes they play an important part in the physiology of the insect, generating the gas that is almost always found in the esophageal diverticulum and also producing an enzyme or other irritating substance which, when injected under the skin of man, causes the inflammation resulting from mosquito bites. Schaudinn considers these yeast cells to play a very important part in the economy of the insect and believes them to be hereditarily passed from the adult through the egg to the larvæ and pupæ.

Mosquitoes fed upon fruits have many more yeast cells in their bodies than those fed upon blood or other material. This we were able to confirm. We also fed mosquitoes upon pure cultures of wild yeasts growing upon banana, and found that the insects fed on such a fermenting diet would soon be so swelled up with gas that their bodies looked like transparent air bubbles. Insects so fed do very badly and it is difficult to keep them alive over a week in tropical temperatures.

Some of these wild yeasts are very interesting; one in particular—the *Saccharomyces apiculatus*, which is found widely spread throughout nature especially on fruit. This particular yeast assumes at times characteristic spindle or lemon shapes, with a bud at the pointed end, somewhat resembling one of the conjugating forms of protozoan organisms with which it has been confused.

We were enabled to isolate this yeast in pure culture from the bananas at Vera Cruz only after some difficulty. The ordinary plate methods failed because the other saccharomyces overgrew the small colonies of *S. apiculatus*. The following expedient finally succeeded: The overripe and fermenting piece of banana containing the morphologic forms desired is planted into orange juice. This culture medium was made by simply squeezing the oranges, taking care not to get any of the oil of the peel, then filtering until clear, and sterilizing by heat in test tubes. As the *Saccharomyces apiculatus* is a bottom yeast, the growth which appears at the bottom of the test tube in twelve to eighteen hours is examined under the microscope and, if the proper forms are found, transferred to another tube containing orange juice. This is repeated until a number of subcultures are made, and as the *Saccharomyces apiculatus* grows better in the orange juice than the other yeasts, the latter are quickly left behind until a pure culture is obtained.

We have noted in stained preparations of these wild yeasts that they sometimes show red chromatin (?) granules in a blue protoplasm

---

[a] Schaudinn, Fritz 1904, Generations- und Wirtswechsel bei *Trypanosoma* und *Spirochaete* (vorläufige Mitteilung.). Arb. a. d. k. Gesundheitsamte, Berl., 4°.

when stained with polychrome methylene blue, such as Goldhorn's. We call atention to this, for isolated yeast bodies of this character stained thus might lead to errors of interpretation.

THE BLOOD IN YELLOW FEVER.

We are fully justified in concluding that in the blood of yellow-fever cases there is a living entity floating free in the plasma and capable of reproducing the disease. The positive results obtained in the filtration and inoculation experiments done by Reed and Carroll, corroborated by the French commission and ourselves, is sufficient proof of that statement.

We carefully examined many blood smears stained with polychrome methylene blue of Wright and Goldhorn, and failed to see the presence of any body which could be considered to stand in any causal relation to the disease.

The smears were taken from 17 cases at periods of five hours to six days after the onset of the disease. In every case blood was taken within the first three days of sickness. In several cases the blood was taken daily or on alternate days. The corpuscles and plasma were carefully searched. The red cells often showed minute blue bodies, usually round and sometimes slightly irregular, which resemble those ascribed to cell degeneration or nuclear rests in anemia.

The mononuclear leucocytes and polymorphonuclear neutrophiles often showed in their protoplasm small, round, clear spaces having a punched-out appearance. These spaces could not be made to take up any one of several stains employed. They were also found in malarial and normal blood.

In making our blood preparations we used a method devised by one of us (Rosenau) about four years ago, which has been in constant use in the Hygienic Laboratory since, and as it has proven so satisfactory in our hands we will describe it. The technique was suggested by the glass slides commonly used for this purpose. The instrument consists of a little glass apparatus we call the "spreader," made by simply welding two pieces of solid glass rods together, as shown in fig. 1. The short arm should be true, so as to lie flat when applied to the slide, and should be several millimeters shorter than the width of the slide. A drop of blood is taken from the ear or finger tip and placed upon one end of the slide in the usual manner. The spreader is then applied to the drop, and if the glass is clean the blood will at once be drawn by capillary attraction across its whole length; it is then spread by a gentle, even stroke, without undue pressure, along the

slide. Very beautiful preparations, with the corpuscles lying singly, are thus obtained.

This little apparatus can readily be made at the blowpipe.

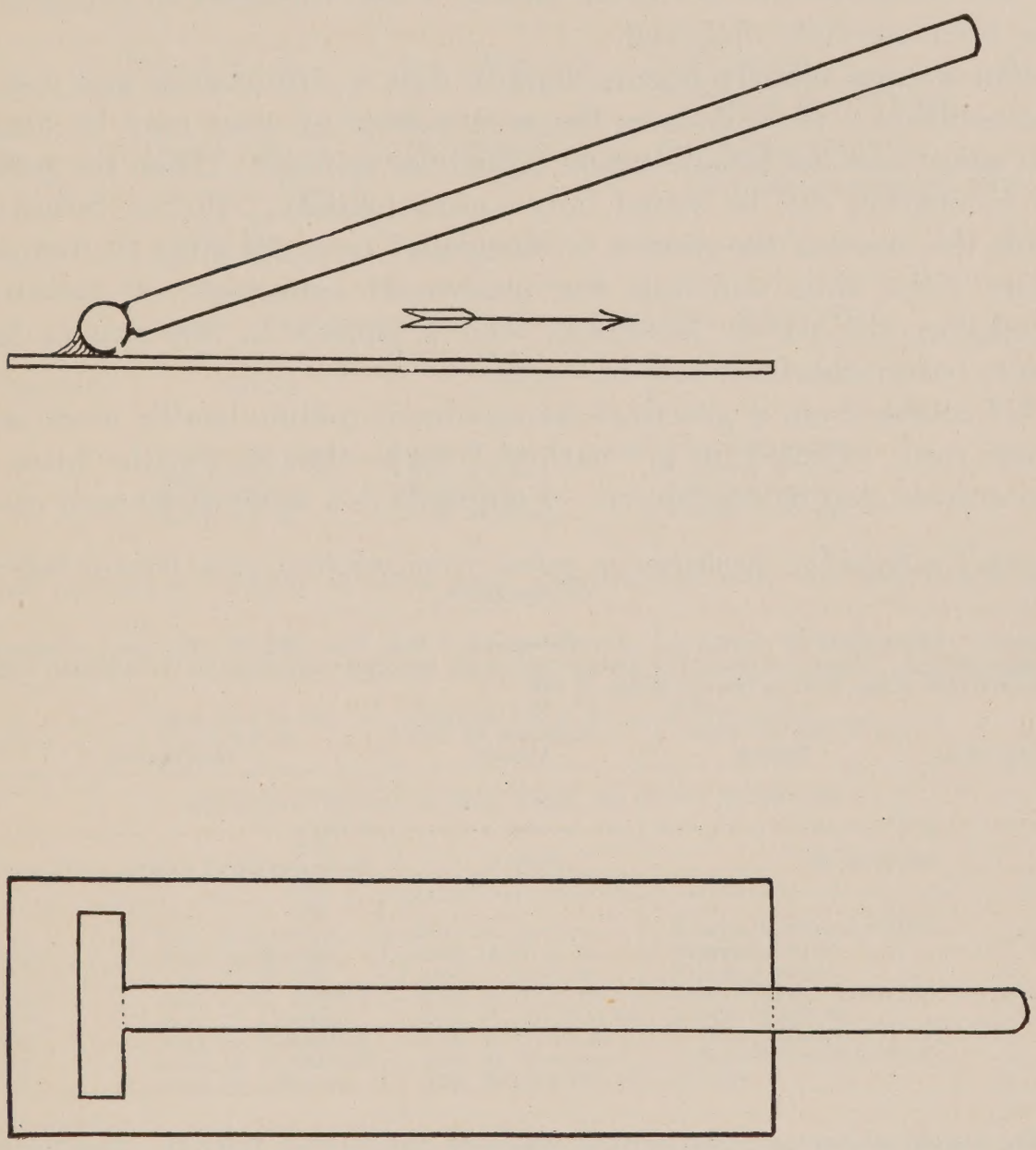


FIG. 1.—Rosenau's spreader for making blood smears.

THE PERIOD OF INCUBATION OF YELLOW FEVER.

The exact period of the incubation of yellow fever is a matter of great importance in quarantine and public-health work. For years the quarantine regulations of the Marine-Hospital Service required a detention of five days, which was considered amply safe to cover the period of incubation of the disease. The regulations of the Public Health and Marine-Hospital Service, promulgated August 10, 1903, lengthened the time of observation in special cases to six days, basing this action upon the recent experimental work which has made it possible to determine the period of incubation of yellow fever with great exactness.

We have collected from the literature all the cases in which the period of incubation may be stated with precision. These are of course for the most part experimental cases in which the exact time of the mosquito bite is known, and in which the onset of the disease has been carefully observed.

The disease usually begins sharply with a chill, pains, and rise of temperature. In such cases the precise hour of onset may be stated, but sometimes the attack begins vaguely or at night. Then the period of incubation can be stated only approximately. In the following table the onset of the disease is considered from the time the temperature rises, thus omitting the prodromal symptoms of lassitude, headache, etc, which sometimes send a patient to bed twenty-four hours before the fever sets in.

Of course from a practical standpoint in public health work only those cases infected in a "natural" way—that is, by the bites of mosquitoes, can be considered. Subjoined is a table of 40 such cases.

TABLE 1.—*Period of incubation in yellow fever, resulting from bites of infected mosquitoes.*

[Carter: "The period of incubation of yellow fever," Med. Rec., Mar. 9, 1901; also, private correspondence. Observations of the disease following one short exposure in the infected region at Orwood, Miss., during the epidemic of 1898.]

Case No.—	Bitten.	Attack.	Incubation.
1			3 days.
2			Do.
3			Between 3 and 4 days.
4			Do.
5			Do.
6			Do.
7			Do.
8			Do.
9			4 days.
10			Do.
11			4½ days.
12			5¼ days.

In these observations, which were of a clinical nature, no attempt was made to determine the period of incubation within hours, anything less than one-fourth of a day being disregarded.

[Reed, Carroll, Agramonte, and Lazear: "The etiology of yellow fever; a preliminary note," Phila. Med. Journ., Oct. 27, 1900.]

Case No.—	Bitten.	Attack.	Incubation.
13 (10)	Aug. 27, 2 p. m.	Aug. 31 (?) a. m.	3 days 17–22 hours (about).
14 (11)	Aug. 31, 11 a. m.	Sept. 6 (?) p. m.	6 days (about). According to the authors, 6 days 2 hours.

TABLE 1.—*Period of incubation in yellow fever, etc.*—Continued.

[Reed, Carroll, and Agramonte: "The etiology of yellow fever; an additional note," Journ. Am. Med. Assoc., Feb. 16, 1901.]

Case No.—	Bitten.	Attack.	Incubation.
15 (1)	Dec. 5, 2 p. m	Dec. 8, 11.30 p. m	3 days 9½ hours.
16 (3)	Dec. 8, 4 p. m	Dec. 14, 9 a. m	4 days 20 hours, took to bed; 5 days 17 hours, onset of fever.
17 (4)	Dec. 9, 10.30 a. m	Dec. 12, 9.30 p. m	3 days 11 hours (about).
18 (5)	Dec. 11, 4.30 p. m	Dec. 15, noon	3 days 19½ hours (bed).
19 (7)	Bitten several times—Dec. 21, noon, and Dec. 22, 4.30 p. m.	Dec. 25, noon	2 days 19½ hours since shortest, and 4 days since longest exposure.
20 (6)	Dec. 30, 11 a. m	Jan. 3, 10.30 a. m	3 days 22½ hours.

[Reed: "Experimental yellow fever," Am. Med., July 6, 1901.]

Case No.—	Bitten.	Attack.	Incubation.
21	Jan. 19, 3.30 p. m	Jan. 23, 3 p. m	3 days 23½ hours.
22	Jan. 31, 9.30 a. m	Feb. 3, noon	3 days 2½ hours.
23	Jan. 6, 11 a. m	Feb. 9, 5 p. m	3 days 6 hours.
24	Jan. 7, 2 p. m	Feb. 10, noon	2 days 22 hours.

[Reed and Carroll: "Etiology of yellow fever; a supplemental note," Am. Med., Feb. 22, 1902.]

Case No.—	Bitten.	Attack.	Incubation.
25 (1)	Sept. 16, 4 p. m	Sept. 19, 4.30 p. m	3 days ½ hour.
26 (2)	Oct. 9, 4 p. m	Oct. 13, midnight	3 days 8 hours (about).

[Guitéras: Revista de Med. Trop., vol. 2, No. 10, 1900–1901.]

Case No.—	Bitten.	Attack.	Incubation.
27 (2)	Feb. 23, 2.45 p. m	Feb. 26, (?) p. m	3 days 10 hours (about).
28 (30)	Aug. 8, 8.30 p. m	Aug. 12, (?) p. m	4 days 5 hours.
29 (31)	Aug. 8, 9.30 p. m	Aug. 11, (?) p. m	3 days 3 hours.
30 (33)	Aug. 9, 9 a. m	Aug. 14, (?) p. m	5 days 3 hours.
31 (37)	Aug. 13, 1.45 p. m	Aug. 17, (?) 8.45 p. m	3 days 19 hours.
32 (39)	Aug. 14, 9 a. m	Aug. 18, (?) 6 a. m	3 days 21 hours.
33 (40)	Aug. 14, 10.15 a. m	Aug. 20, forenoon	5 days 21 hours (about).
34 (41)	Aug. 22, 4.30 p. m	Aug. 25, (?) 4.30 p. m	3 days.

[Parker, Beyer, and Pothier: "A study of the etiology of yellow fever," Yellow Fever Institute, Bull. No. 13, March, 1903.]

Case No.—	Bitten.	Attack.	Incubation.
35	Sept. 4, 9.30 a. m	Sept. 7, (?) a. m	3 days 2 hours (about).

[Marchoux, Salimbeni, and Simond: "La fievre jaune," Rapport de la Mission Francaise, Institut Pasteur, Annales, November, 1903.]

Case No.—	Bitten.	Attack.	Incubation.
36 (2)	----------	----------	3 days 18 hours.
37 (17)[a]	----------	----------	3 days 22 hours.
38 (20)	----------	----------	5 days 22 hours.
39 (22)[b]	----------	----------	7 days 5 hours.

[a] Before being bitten by infected mosquitoes patient had received injections of blood from yellow fever cases to induce immunity: 5 cc. blood twelve days old; followed fifteen days later by same quantity eight days old.

[b] This man previously had been given 20 cc. serum taken on the eighth day and passed through a Berkefeld filter; six days later 20 cc. of same serum not filtered; subsequently bitten by infected mosquitoes. The serum injections may have induced a partial immunity, which delayed the onset and modified the disease, for he had a mild attack.

TABLE 1.—*Period of incubation in yellow fever, etc.*—Continued.

[Francis and Beyer.]

Case No.—	Bitten.	Attack.	Incubation.
40	Sept. 11, 9 a. m., and Sept. 12, 2.30 p. m.	Sept. 14, 3.30 p. m.	3 days 7 hours, or 2 days 1 hour.

A study of the 40 cases in this table discloses the fact that yellow fever usually begins about three days after the mosquito bites.

The period of incubation resulting from this natural method of conveying the disease is rarely under three days. We have but one such authentic instance, namely, two days twenty-four hours (case No. 24).

The longest period observed was seven days five hours, but it must be noted that the man who had this unusually long period of incubation had previously been treated with injections of immunizing sera, which may have delayed the onset and modified the disease, for he had a mild attack.

Leaving this case (No. 39) out of consideration, the longest period of incubation resulting from the bites of mosquitoes is the case (No. 14) of Reed, Carroll, Argamonte, and Lazear, in which an incubation period of six days two hours was observed. This corresponds strikingly to Carter's clinical observations in which he reports a case with an incubation period of five and three-fourths days. See case No. 12 in Table 1.

The French commission, working in Rio de Janeiro, came to the concluson that the period of incubation of the disease may be much longer than this; but we find on analyzing their work that they drew their inferences largely from the disease produced by such artificial means as the inoculation of modified blood serum.

One of the conclusions of this commission was that yellow fever may not infrequently incubate for twelve days before symptoms declare themselves.

They state that "this incubation of twelve days is not absolutely rare. We have had occasion to see that the natural infection may also present an incubation equally long."

With this statement we must take issue, for the long experience of the Public Health and Marine-Hospital Service in the many wars it has waged against yellow fever has amply demonstrated that for practical purposes five days is sufficient to cover the period of incubation of the great majority of cases. An analysis of all the cases reported in Table 1 supports this view.

The French Commission reports several cases in support of their contention. One, a young man 18 years old, who took yellow fever ten days after having arrived in Petropolis from Rio de Janeiro. Petropolis is a village free from yellow fever. Another instance

was a girl 12 years old, who was taken with yellow fever ten days after returning from Rio, her father having sent her to Petropolis because his wife and three other children had the fever.

We do not doubt that Petropolis is "indemne," free of *Stegomyia fasciata*, and that the disease has never been known to spread there; but the communication with Rio is close, and if yellow fever cases are brought to Petropolis it is conceivable that infected mosquitoes may also be carried. There are many other "loopholes" which weaken observations of this kind, and we have therefore refrained from placing them in our table.

The last case cited by the French commission is as follows:

On board the vessel *Messageries*, returning to Europe, having taken passengers from Rio de Janeiro, an isolated case of yellow fever declared itself among the latter passengers between Dakar and Lisbonne; that is, nine to fourteen days.

It was our experience that some cases of yellow fever are so mild that they are detected with difficulty, especially under such unfavorable conditions as on board ship. "The isolated case" on board the *Messageries* may have been the second case, especially as the fourteen days is sufficient to cover the "extrinsic incubation" of the disease. The literature has several instances of such cases. They should be carefully considered before drawing definite conclusions.

It is interesting to compare the period of incubation resulting from exposure to infection in the "natural" way with the period of incubation resulting from experimental yellow fever, produced by the inoculation of blood or blood serum. The following table shows 17 such cases:

TABLE 2.—*Period of incubation in yellow fever, resulting from the injection of blood.*

[Reed, Carroll, and Agramonte: "Experimental yellow fever," Am. Med., July 6, 1901.]

Case No.—	Inoculated.	Attack.	Incubation.
1	Jan. 4, 11 a. m	Jan. 8, 9 a. m	3 days 22 hours.
2	Jan. 8, 9 a. m	Jan. 11, 9 a. m	2 days 12 hours.
3	Jan. 22, 1 p. m	Jan. 24, 9 a. m	1 day 19 hours.
4	Jan. 25, 12.45 p. m	Jan. 28, 1.15 p. m	3 days 1 hour.

No. 1 received subcutaneously 2 cc. blood taken on second day.
No. 2 received subcutaneously 1.5 cc. blood taken 12 hours after beginning of attack.
No. 3 received subcutaneously 0.5 cc. blood taken on second day.
No. 4 received subcutaneously 1 cc. blood taken 27¼ hours after commencement of disease.

[Reed and Carroll: "The etiology of yellow fever; a supplemental note," Am. Med., Feb. 22, 1902.]

Case No.—	Inoculated.	Attack.	Incubation.
5 (3)	Oct. 15, 4 p. m	Oct. 20, 6 p. m	5 days 2 hours.

Case 5 received subcutaneously 0.75 cc. partially defibrinated blood 15½ hours old.

TABLE 2.—*Period of incubation in yellow fever, etc.*—Continued.

[Reed and Carroll: "The etiology of yellow fever; a supplemental note," Am. Med., Feb. 22, 1902.]

Case No.—	Inoculated.	Attack.	Incubation.
6 (7)	Oct. 15, 11 a. m	Oct. 19, 3 p. m	4 days 4 hours.
7 (8)	Oct. 15, 11.05 a. m	Oct. 19, noon	4 days 1 hour.

Cases 6 and 7 were inoculated subcutaneously with 3 cc. of an equal volume of water and serum filtered through a Berkefeld filter.

[Marchoux, Salimbeni, and Simond: "La fievre jaune," Rapport de la mission française, Institute Pasteur, Annales, November, 1903.]

Case No.—		Incubation.
8 (1)	1 cc. serum	5 days 5 hours.
9 (3)	5 cc. serum heated to 55° for ten minutes; five days later, 10 cc. heated to 55° for ten minutes; seven days later, 1 cc. blood. This was a "remarkably benign case," and as the man had been injected previously with heated yellow fever serum, the immunity produced probably explains the long period of incubation as well as the mildness of the attack.	12 days 12 hours.
10 (4)	5 cc. serum heated to 55° for twenty minutes; seven days later, 10 cc. serum heated to 55° for ten minutes; eight days later, 1 cc. serum heated to 55° for five minutes. Then 1 cc. serum. The same explanation for this unusually long period of incubation as above, especially as a parallel case similarly treated showed an immunity.	8 days 5 hours.
11 (7)	1 cc. serum filtered through a Chamberland F filter	5 days 18 hours.
12 (8)	do	12 days 18 hours.
13 (13)	0.1 cc. (1 drop) of serum	4 days 18 hours.
14 (15)	5 cc. blood, 5 days old	2 days 21 hours.

[Francis and Beyer.]

Case No.—	Inoculated.	Attack.	Incubation.
15 (2) [a]	Sept. 15, 4 p. m	Sept. 17, (?) a. m	1 day, 15 hours (about).
16 (3) [b]	do	do	Do.
17 (4) [c]	do	Sept. 17, 9 a. m	1 day, 17 hours.

[a] Intravenous injection of 1.75 cc. serum diluted with an equal volume of salt solution and filtered through a Chamberland B filter.
[b] Intravenous injection of 2.5 cc. serum diluted with an equal volume of salt solution and filtered through a Chamberland B filter.
[c] Intravenous injection of 2.5 cc. serum diluted with an equal volume of salt solution and filtered through a Chamberland B filter.

It will be noted from these 17 cases that the period of incubation of yellow fever produced by the inoculation of blood or blood serum is not so constant a factor as in Table 1, in which the disease was induced by the bites of mosquitoes.

The shortest time in this table is one day fifteen hours, and the longest twelve days eighteen hours.

Surg. H. R. Carter, Public Health and Marine-Hospital Service, has given special attention to this phase of the subject, and we are indebted to him for valuable suggestions.

THE FILTRATION OF YELLOW-FEVER BLOOD.

Reed and Carroll (Am. Med., Feb. 22, 1902) were the first to filter yellow-fever blood and prove the infectiousness of the filtrate. They passed it through a Berkefeld filter, which on testing held back the *Staphylococcus pyogenes aureus.*

The filtrate showed no growth in bouillon, and yet when injected into nonimmunes produced yellow fever. The blood from the latter was also shown to be capable of producing yellow fever when injected into a third subject.

That the men inoculated with the filtrate suffered from yellow fever induced by a morphologic entity which passed the filter, and not from a toxemia, was shown not only by their rather long periods of incubation, but was conclusively shown by carrying their experiment to the third degree.

The following experiments were planned in order to determine among other things whether the organism of yellow fever, as it exists in the blood serum, is capable of passing the pores of the Pasteur-Chamberland B filter:

An investigation of the literature of the other filterable viruses shows that the South African horse sickness is the only one which has yet been reported as having passed the Chamberland B filter.

In the filters of the Pasteur-Chamberland system those marked "B" are finer, more compact, with thicker walls, and consequently less porous than those marked F. We have been informed by Assistant Surgeon-General H. D. Geddings, who has recently inquired about this in Paris, that only two grades—B and F—are now being made.

The subjects used for our experimentation were all volunteers, nonimmunes, and carefully selected from among the native Mexicans at Jalapa and the adjacent mountainous country, taken by train to Vera Cruz, and immediately placed within the screened wards of our hospital. All cases recovered.

Jalapa is a town having an elevation of about 4,000 feet, where yellow fever has never been known to spread and has not existed, except for the cases occasionally imported from the coast (tierra caliente).

In order that the case from which we drew the blood for filtration should be one in which there was the highest degree of confidence as to the diagnosis of yellow fever, we decided to produce the disease through the bites of infected mosquitoes rather than to select a case by clinical evidence alone from the yellow-fever wards.

Mosquitos which had been allowed to feed upon typical cases of yellow fever in San Sebastian Hospital, Vera Cruz, were applied in succession to the hands of four persons whom we had selected as being nonimmunes. The first three failed to become infected, but the fourth took sick with what proved typical yellow fever. The histories of the three negative cases are here given in brief:

G. M., age 22, Mexican.—On August 13 he was taken to Vera Cruz and placed in our screened ward. August 15, at 3.20 p. m., he was bitten by two mosquitoes which had fed twelve days previously, at 9.30 a. m., on J. R., a fatal case of yellow fever. Nothing unusual

was noticed in the patient during the period of observation, which continued until August 28.

J. O., age 18, Mexican.—He was taken to Vera Cruz August 13 and placed in the mosquito-proof ward. On August 28, at 9.30 a. m., he was bitten by four mosquitoes, two of which had fed sixteen days previously, at 4 p. m., on A. L., a fatal case of yellow fever, forty hours after the onset of the disease, and the other two had fed fifteen days previously, at 10.30 a. m., on the same case fifty-eight hours after the onset of the disease. The patient remained perfectly well throughout the following month while under observation in the screened room.

M. R., age 21, Mexican.—He was brought to Vera Cruz August 28 and kept in the screened ward. On September 1, at 6.30 p. m., he was bitten by two mosquitoes which had fed nineteen days before, at 10.30 a. m., on A. L., a fatal case of yellow fever, fifty-eight hours after the onset of the disease. The patient continued in his usual health during Sepember 2 and 3. On September 4, at 2 p. m., on going into the ward the patient was found wrapped in his blanket and said he felt chilly and complained of slight temporal headache. There was no elevation of temperature.

The next case succeeded:

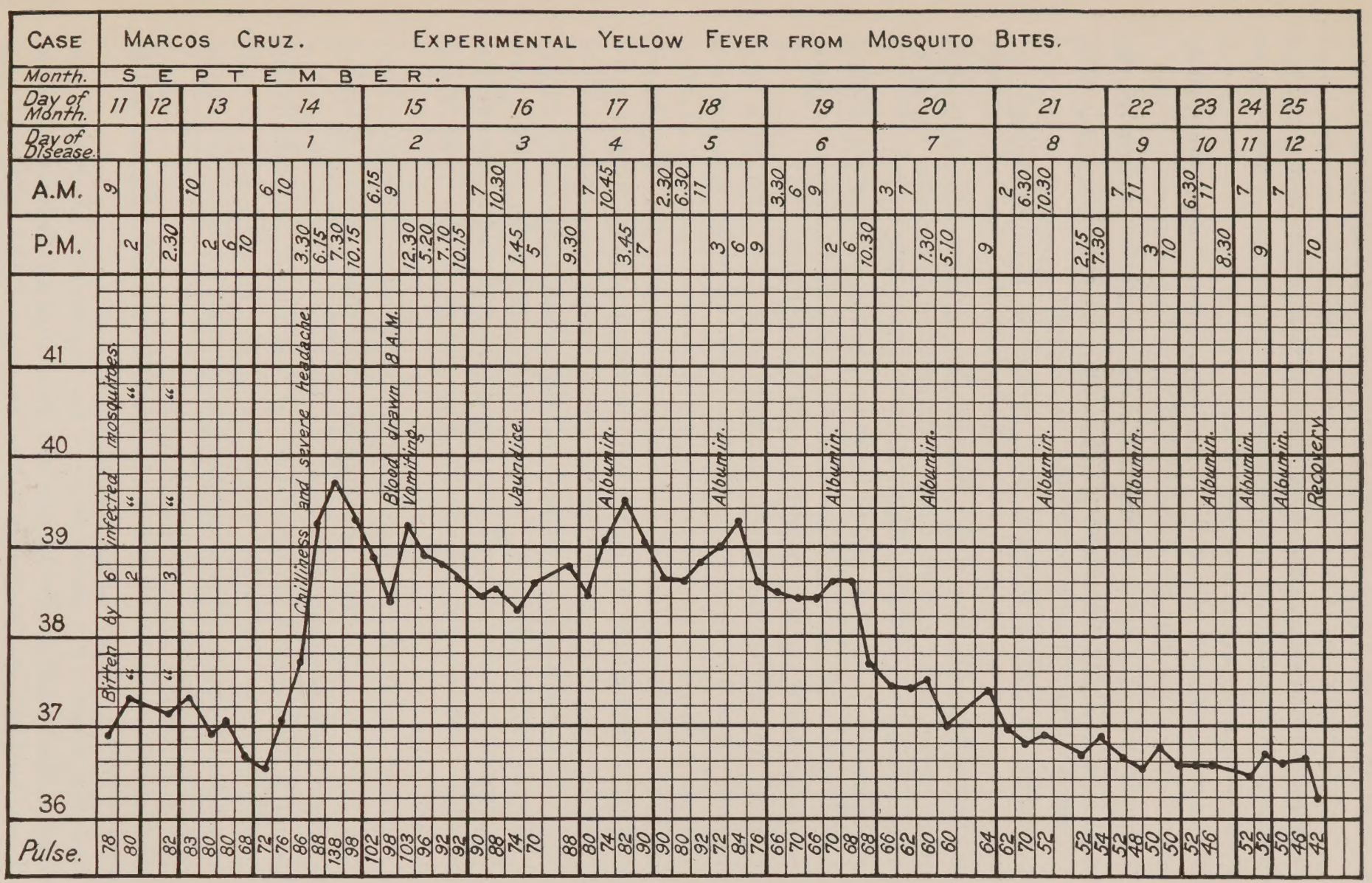
Yellow Fever Produced by the Bites of Mosquitoes.

Marcos Cruz (case XLII), age 21, born in Perote, a mountain town free from yellow fever, where he has always lived. States that he never had fever of any kind. He was physically sound on examination, and brought to Vera Cruz, where he was immediately placed in a mosquito-proof room and kept under observation for fourteen days, when he was bitten by 11 mosquitoes, as follows:

On September 11, at 9 o'clock a. m., 3 mosquitoes which had fed fifteen days previously, at 9.30 a. m., upon Trinidad Martinez, a fatal case, fifty-one hours after the onset. At the same time, 3 other mosquitoes, which had fed fourteen days previously, at 2.30 p. m., upon Hipolito Vasquez, a fatal case, sixty-nine hours after the onset of the disease. At 2 p. m. of the same day he was bitten by 2 more mosquitoes, which had fed fourteen days previously, at 2.30 p. m., on Hipolito Vasquez, sixty-nine hours after the onset.

The next day, September 12, at 2.30 p. m., he was bitten by 3 mosquitoes, 2 of which had fed fifteen days previously, at 2.30 p. m., on Hipolito Vasquez, sixty-nine hours after the onset of the disease, and the other had fed sixteen days previously, at 9.30 a. m., on Trinidad Martinez, fifty-one hours after the onset of his disease.

On September 12 and 13 the patient had no symptoms, and his temperature remained normal.



Temperature chart of Marcos Cruz.

September 14, at 4 p. m., Cruz complained of feeling chilly and had frontal headache. The chilly sensation lasted for several hours and the headache became so severe that he was given ice caps to his forehead for the relief of this symptom. The conjunctivæ became injected; the gums turgid and red.

The case continued clinically with typical symptoms of yellow fever.

On September 15, at 8 a. m., there was a slight icterus of the eyes; at 10 a. m. there was marked vomiting; at 7 p. m. the gums were very much swollen. Urine contained no albumin.

September 16: Eyes injected and yellowish, skin jaundiced; no albumin.

On September 17, the third day of the disease, albumin first appeared in the urine and was found daily until September 25, after which examination was discontinued.

On September 15, at 8 a. m., 16 hours after the onset of the disease 80 cc. of blood were drawn into a sterile flask by means of an aspirating needle from the median basilic vein of his left arm.

The flask was set aside in the lower part of the ice chest, at a temperature of from 16° to 19° C., for five hours in order to allow the blood to coagulate.

Thirty-five cubic centimeters of the clear serum were then drawn off, and to it was added an equal volume of physiological salt solution.

The mixture was transferred with all due precautions to the inside of a Pasteur-Chamberland B bougie and filtered from within outward by means of vacuum, which was applied to the outer surface of the filter in a reverse manner to that shown in fig. 2, omitting the paraffin cup.

It required one hour to obtain 13.5 cc. of filtrate, which was used for the injection of three nonimmunes, Bonifacio Orea, German Ramos, and Guadalupe Gomez.

Orea and Ramos each received 5 cc. of the filtrate, injected intravenously into one of the veins of the arm. As the same was diluted with an equal volume of salt solution, each man received 2.5 cc. of the original blood serum.

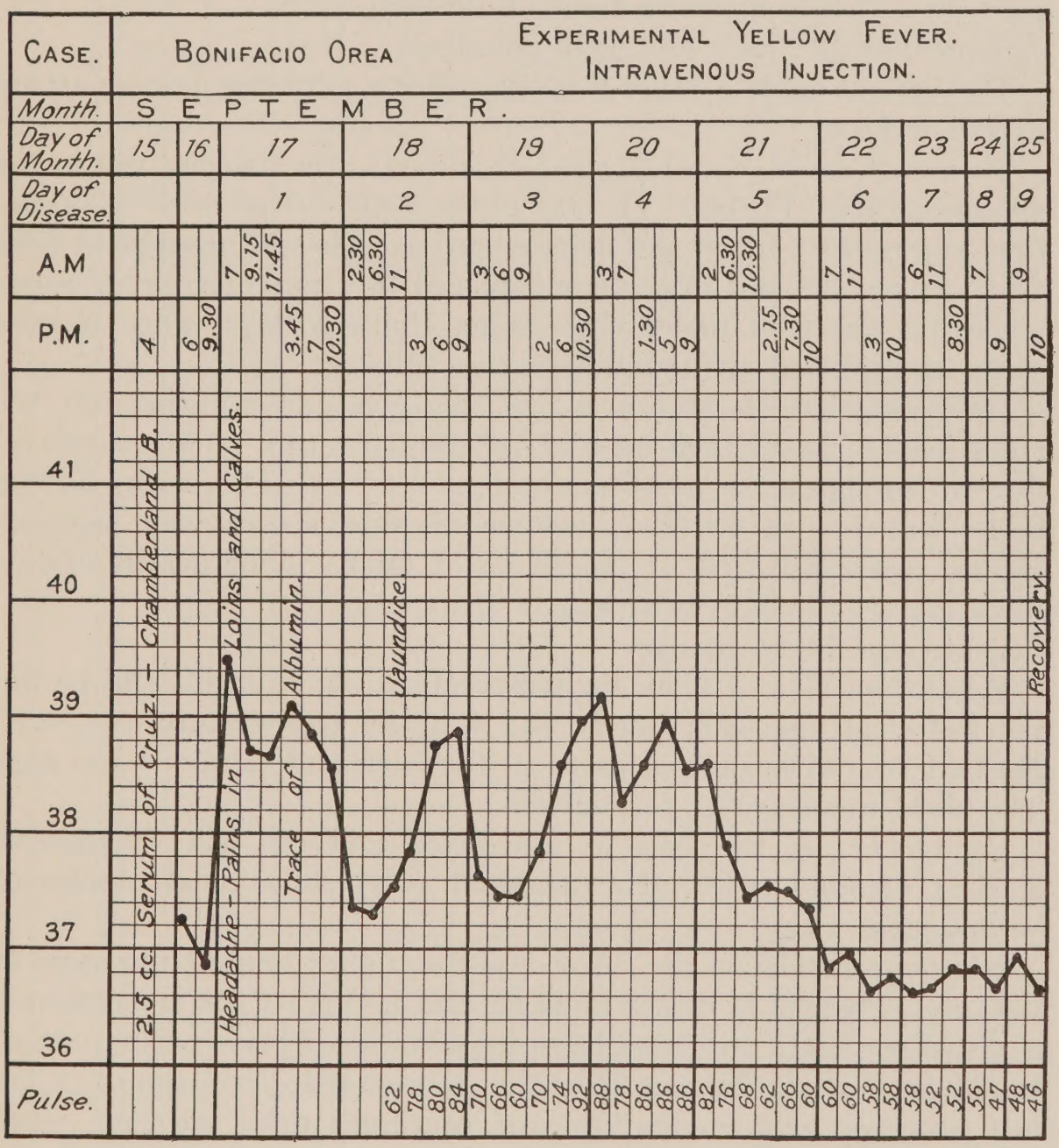
Gomez received 3.5 cc. of the mixture, representing 1.75 cc. of the original serum.

These injections were made at 4 p. m. September 15, which was just eight hours after the blood had been drawn from Cruz, the blood having kept five hours at a temperature of 16° to 19° C. and three hours at room temperature.

Yellow Fever Produced by the Inoculation of Blood Serum.

Bonifacio Orea (case LXV), aged 33, single; born in the mountains near Puebla; said that he had never been on the coast and never had any sickness of any kind. He was physically sound on examination; blood and urine negative.

He was brought to Vera Cruz August 28 and kept in a mosquito-proof room under observation for eighteen days.



Temperature chart of Bonifacio Orea.

On September 15, at 4 p. m., he was given an intravenous injection into one of his arm veins of 5 cc. of Marcos Cruz's diluted serum filtered through a Chamberland B bougie. As the serum had been diluted with an equal quantity of physiological salt solution Orea

actually received 2.5 cc. of the blood serum of Cruz. (For details of this filtration, see p. 62.)

On September 16 there were no symptoms.

September 17, at 7 a. m., he was found with a temperature of 39.5° C., with marked frontal headache, pains in the loins and calves of the legs. His gums were slightly swollen and he had injection of the ocular conjunctivæ. The patient said that at 1 o'clock in the morning he felt like stretching and had a chill. The blood showed no malarial parasites. Urine showed a trace of albumin.

September 18: Eyes and body jaundiced.

The patient made a rapid recovery after a mild but definite attack of yellow fever.

German Ramos (case L), aged 22, single; has always lived in the mountains near Puebla. He was given a careful physical examination August 26. Blood and urine negative. He was brought to Vera Cruz August 28 and immediately placed in a mosquito-proof room, where he was kept under observation eighteen days, when he was given an injection of filtered serum, as follows:

On September 15, at 4 p. m., he was given an injection into the right median basilic vein of 5 cc. of diluted serum of Marcos Cruz, filtered through a Pasteur-Chamberland filter B. This serum having been diluted with an equal volume of physiological salt solution, Ramos received 2.5 cc. of the serum of Cruz. (For details of this filtration, see the records of Marcos Cruz, page 62.)

September 16, no change.

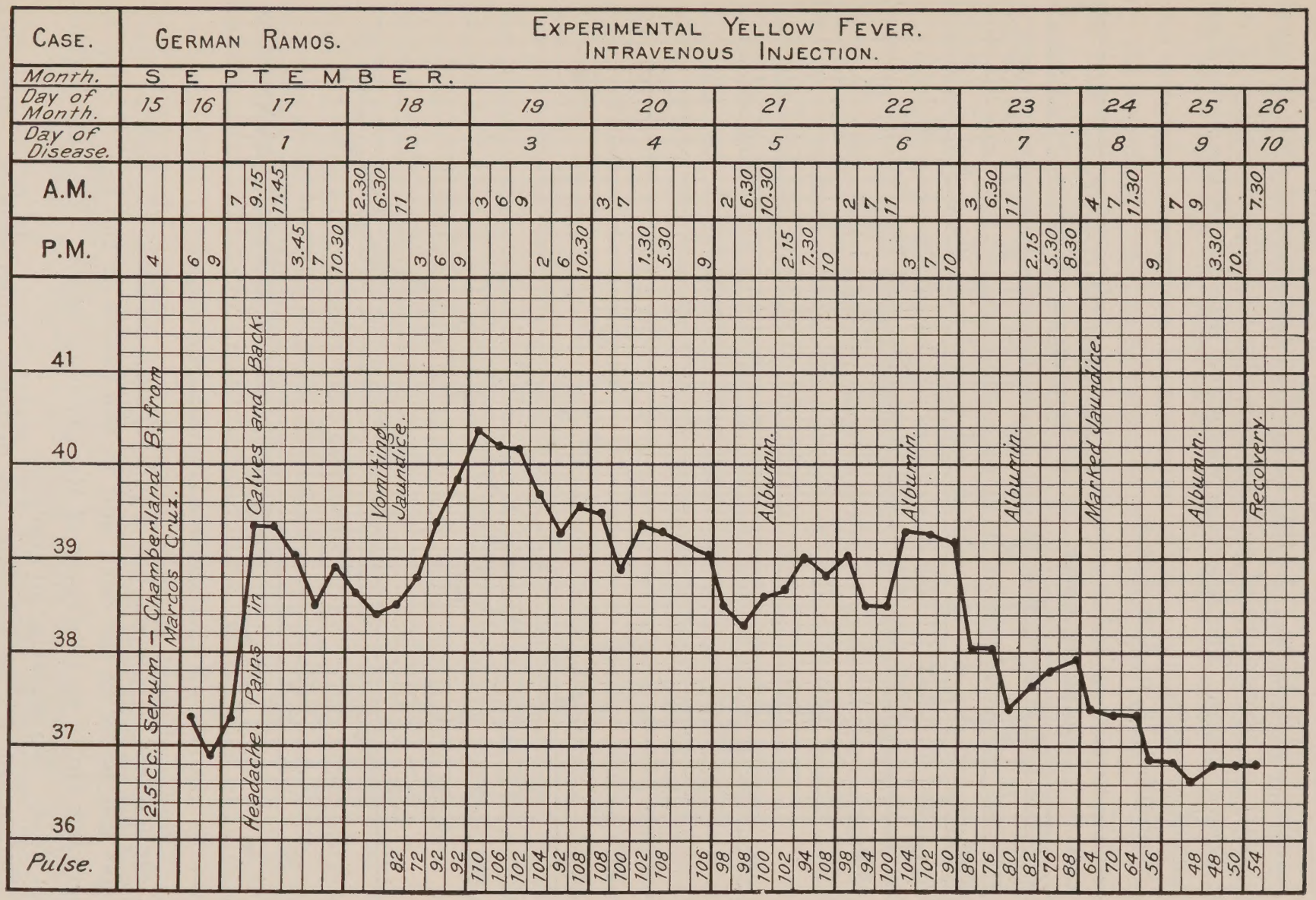
September 17, at 7 a. m., his temperature was 37.3° C., and he did not feel at all unusual; but at 9.15 a. m. his temperature was 39.4°. He had frontal headache, pains in the back and calves of the legs. The blood was negative for malaria.

September 18, the eyes were injected and the body jaundiced. Patient vomited twice. He exhibited a typical clinical picture of yellow fever.

His temperature on the third day was higher than at the onset of the disease, which is rather unusual, and in Vera Cruz is considered a grave sign. It will also be noted that his febrile period, which lasted seven days, did not show the characteristic remission. Albumin first appeared on the 21st and continued until the 25th, when examination was discontinued.

Patient recovered.

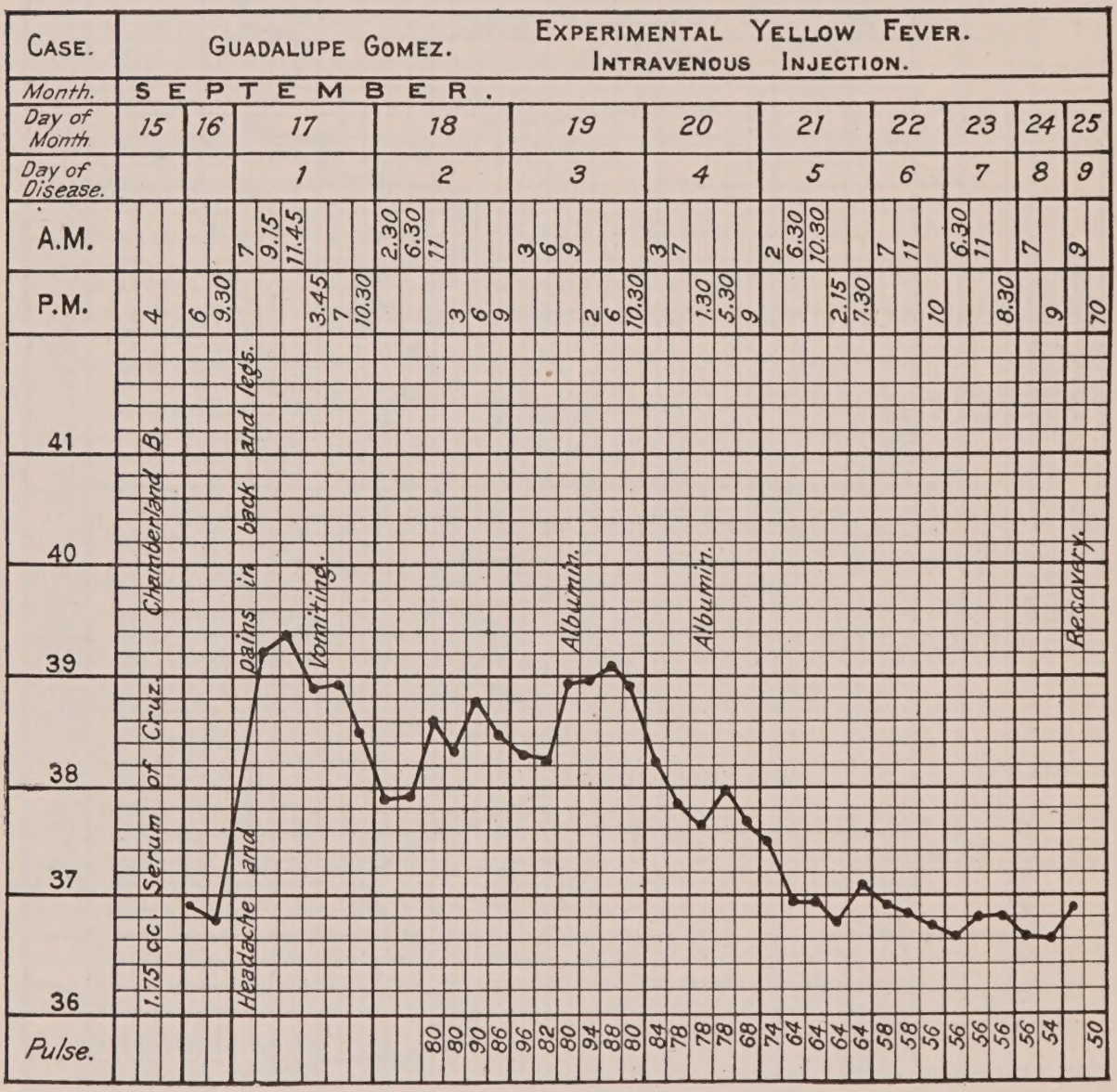
Guadalupe Gomez (case LI); born in Jalapa, aged 17, had never been on the coast, and states that he never had any kind of fever. He was physically sound when examined; blood and urine negative. He was brought to Vera Cruz August 28 and placed in a mosquito-proof room, where he was kept under observation eighteen days.



Temperature chart of German Ramos.

On September 15 at 4 p. m. he was given an injection of 3.5 cc. of diluted blood serum from Marcos Cruz, filtered through a Pasteur-Chamberland B filter. As this serum had been diluted with an equal quantity of physiological salt solution, he actually receved 1.75 cc. of Cruz's serum. The injection was given into one of the superficial veins of the arm.

September 16, no symptoms.



Temperature chart of Guadalupe Gomez.

September 17, 7 a. m., had a temperature of 38.1° C., frontal headache and pains in the back and in the calves of the legs. Says he first felt chilly at 10 o'clock of the previous night Vomited yellowish fluid. No malaria, no albumin.

September 18, no albumin, no malaria, gums swollen. Bleeding from right nostril.

September 19 and 20, urine shows albumin, but none subsequently.

Patient made a rapid and uneventful recovery.

REMARKS ON FILTRATION EXPERIMENTS WITH YELLOW-FEVER BLOOD.

We have been careful to give with some minuteness all the details of the manner in which the blood was filtered in these experiments. We know that the filtration of micro-organisms or other small particles through porcelain or diatomaceous earth is influenced very much by the length of time the filtration is continued, the pressure used, by the character of the fluid in which the particles are suspended, the temperature, and other factors which are perhaps less known.

Our review of the literature on the filtration of blood and body juices containing the infectious material of diseases, the causes of which are unknown and which are believed to be ultramicroscopic, disclosed reports of successful and unsuccessful filtration with such meager details that it is difficult to draw proper conclusions. Those factors which control the power of a given filter to allow an organism to pass or to hold it back also account for the different results which various experimenters have obtained in certain cases.

For instance, we succeeded in passing diluted yellow-fever serum through the closest-grained Pasteur-Chamberland B filter that we could obtain, whereas the French commission—Marchoux, Salimbeni, and Simond—working at Rio de Janeiro, failed to pass the infective agent of yellow fever through a Chamberland B filter, though they found that it did pass through the Chamberland F filter,. As the French commission used undiluted blood and we used diluted serum, the apparent discrepancy in results is accounted for; for it is a well-known fact that particles suspended in an albuminous medium filter with more difficulty than particles suspended in water, alcohol, or other limpid menstra of this character.

Nocard, Roux, and Dujardin-Beaumetz, in 1899, endeavored to repeat Löffler's experiment with aphthous fever. They first failed to pass the infective agent contained in the lymph of this disease through a Berkefeld filter because they used an albuminous fluid, viz, "Martin's serum-bouillon," in order to dilute the lymph with a nutrient medium, thus hoping to obtain cultures without the danger of contamination in the filtrate. They found, however, that the albuminous matter contained in the diluting fluid clogged the pores of the filter, so that the filtrate was not virulent.

They repeated the experiment, using water to dilute the lymph in the proportion of 1 to 50, when they found the organisms causing aphthous fever readily passed through a Berkefeld filter, and gave the disease by intravenous injection to young and old cattle.

It is also a well-known fact that filters which successfully hold back certain bacteria will permit them to pass if the filtration is con-

tinued long enough. In this case the organisms are believed to grow through the pores of the filter.

It is also evident that the passage of small particles through the pores of a filter depends directly upon the pressure used, and in all filtration experiments the exact pressure, whether positive or negative, should be stated.

It is further necessary to call attention to the very great discrepancy in filters. We have made a careful study of various filters found upon the market and find that there is no satisfactory method by which they can be accurately graded, although we find in certain makes an attempt to graduate the power of the filter. Filters of course should always be tested under water with air pressure for pin holes and cracks, and also with small bacteria for permeability. It is only in this way that we may determine approximately what a particular filter is capable of doing.

The Berkefeld filters, made of diatomaceous earth, are more porous and variable than the Pasteur-Chamberland bougies, made of unglazed porcelain, which have finer pores and are more constant in their ability to filter micro-organisms.

After filters have been tested they must be dried and sterilized with the greatest care in order to prevent cracking, and should always be tested for porosity with microbes after filtration in order to insure this point.

We have found in testing various filters that the weakest part is apt to be the joint, and that in any mechanical arrangement of the filter and flask there is the greatest danger of contamination and untrustworthy results if either the liquid that is being filtered or any other fluid comes in contact with a joint.

It will be noted that in the arrangement which we had for filtration by pressure (fig. 2), the fluid was simply passed into the Pasteur-Chamberland candle and withdrawn by means of a pipette in such a manner that contact between the two fluids was eliminated, and that no dependence was placed upon the security of any joints except those necessary to retain the air pressure. In the case of the small Berkefeld filter (fig. 3), in which the filtration was done by the pressure of the atmosphere produced by a vacuum, the joint between the filtering candle and the metal top was kept well out of the liquid, so that here again was avoided the possibility of error from this source.

TESTING OF FILTERS WITH OBJECTS OF "ULTRAMICROSCOPIC" SIZE.

Four filters of the Pasteur-Chamberland system, letter B, were tested to determine whether they would allow particles of microscopic size to pass into the filtrate.

At the outset we were confronted with the difficulty of finding a

substance suitable for such a purpose. The substance should be in a very fine state of division, composed of particles grading gradually in size from the ultramicroscopic to those of definite microscopic size The substance should be insoluble in the menstrum, so that a particle recognized in the filtrate would not represent a precipitate formed after passing the filter.

We finally selected carbon on account of its insolubility, the very fine state of division into which it can be brought, and the ease with which the small particles may be recognized because of their black color and violent Brownian movement.

To 60 cc. of distilled water we added .40 drops of Higgins's American india ink, bought on the market, and this suspension was placed into each of the four Chamberland B filters and drawn through the walls from within outward by a vaccuum in a reverse manner to fig. 2. The first water to come through was pale, but gradually it became slightly brown and later the surface of the filter took on a distinctly dark color. About one and a quarter hours was required for the 60 cc. to pass through each filter.

The filtrates of the four filters were examined with Zeiss microscopes, using objectives of 1.5 and 2 mm. and oculars 4 to 12, and there was not the slightest difficulty to see in the hanging drops small particles of carbon in active Brownian movement. Dried specimens of the filtrate showed small particles plainly visible.

These filters were new and were tested under pressure beneath the surface of water and found free from cracks and pin holes. Before testing with the india ink they were washed with distilled water, about 200 cc. being put through each one.

The filters first became black in disseminated points on the surface. The black areas were of irregular shape, having a diameter of one-eighth to one-fourth inch. As the filtration continued these areas became larger. At the end of the filtration the filter had a distinctly mottled appearance, showing streaks of white, small circumscribed areas of deep black, and larger areas less deeply stained. That these areas of black are carbon may be demonstrated by burning them in the flame.

This shows a lack of uniformity in the structure of each individual filter, which only confirms what may be seen after breaking a filter into pieces. At places air spaces may be seen which may extend through almost the entire thickness of the wall, thus reducing the real thickness of the filter to a mere shell.

Two new Berkefeld filters, $2\frac{1}{4}$ by $\frac{5}{8}$ inches, were tested with the dilute ink solution. Neither pressure nor vacuum was used. The filtrate came in drops in rapid succession and was as black as the test fluid and showed the particles of carbon under the microscope.

These same filters were tested with a bouillon culture of *Staphylococcus pyogenes aureus* in a reverse manner to fig. 2. From three to four hours were taken in passing 150 cc. of the bouillon culture through each filter. The filtrates remained sterile after twelve days in the incubator.

The Berkefeld filters were rigged with the glass cylinders which come with them and a vacuum was used. About four hours were required to run 150 cc. of the bouillon culture through each filter.

THE FILTRATION OF CERTAIN VIRUSES.

Peripneumonia of cattle, rinderpest, foot-and-mouth disease, South African horse sickness, exudative typhus of chickens, mosaic disease of the tobacco leaf, yellow fever, epithelioma contagiosum of fowls, hydrophobia, clavelee, and hog cholera have each been shown to be due to a virus which passes the pores of certain porcelain and diatomaceous filters which hold back the ordinary bacteria.

With the exception of peripneumonia we know nothing of the character of the infective agent in these filtrates, which by direct microscopic examination and by cultural methods have yielded no morphological entity.

The outlook for finding in the body fluids, the specific cause of any one of these diseases, by the microscopes in present use is encouraging as long as we can say that we have a filter which will not allow the virus of the disease to pass, but which does allow the particles of some test substance to be plainly seen in the filtrate.

It is far more important to know what particular filter a certain virus can *not* pass through than it is to know what brands of filter it does pass through. Given a filter that will not permit the virus of a disease to pass through its pores, and if on testing that filter we find that we can put through it visible particles of some test substance, there is plenty of hope that the infective agent of that virus may be visible with our present oil-immersion systems.

On the other hand, if we can find a filter which will not transmit particles of microscopic size and yet will allow the virus of a certain disease to pass into the filtrate, we can not expect to see the individual entities in the virus.

Several factors influence the filterability of a virus, namely, the kind of filter used, the character of the menstruum in which the virus is suspended, the degree of pressure or vacuum used, the amount of time allowed to the process, the temperature, the motility of the particles, and other factors. Unfortunately, in the study of the literature of the various filterable viruses we sometimes fail to find exact data on all these points.

Peripneumonia.—Peripneumonia [a] of cattle is the only filterable virus which has so far given a visible growth on artificial media. Collodion sacs were filled with Martin's peptone bouillon, to which was added a little serum of the rabbit or cow in the proportion of 1 : 20. The sac was then inoculated with peripneumonia and placed in the peritoneal cavity of rabbits and cows for fifteen to twenty days. The fluid became turbid and in it, under a magnification of 2,000 diameters, could be seen the most extremely small, moving, strongly refractile points. In a series of subcultures made from such a growth the last of the series was virulent.

The colonies on agar mixed with bouillon-serum, were transparent, small, and made up of exceedingly fine particles whose form it was impossible to determine. The microbe of this disease was made to pass the Berkefeld and Chamberland F filters, but when an albuminous diluting liquid was used, it could not be made to pass either.[b]

Foot-and-mouth disease.—Loeffler and Frosch [c] state that lymph was taken from the blebs of calves suffering with this affection, diluted with 35 parts of water, and then passed through a filter candle. The filtrate, in amounts which correspond to one-tenth to one-fortieth cubic centimeter of the original lymph, when injected into calves caused them to sicken in two days, the same as the control animals into which were injected equal amounts of unfiltered fluid.

McFadyean says that foot-and-mouth disease passes the Berkefeld filter when in watery suspension, but is arrested when in an albuminous fluid.

Nocard [d] says that aphthous fever passes through Berkefield, Chamberland, and Kitasato filters.

South African horse sickness.—Nocard [a] succeeded in passing the virus of this disease through a Berkefeld filter only.

McFadyean [e] reports that pure blood taken from an animal sick with the disease was passed through the Berkefeld filter under a pressure of 26 inches of mercury and the filtrate, when inoculated into a horse, produced the disease.

When the blood serum was diluted with four parts of water and

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[a] Nocard, Roux, Borrel, Salimbeni et Dujardin-Beaumetz: Le microbe de la peripneumonie. Ann. de l'Inst. Pasteur, vol. 12, 1898, p. 240, etc.

[b] Nocard, Roux, and Dujardin-Beaumetz: Etudes sur la peripneumonie. Recuil de med. vet., 1899, 8e. serie, Oct. 26, 1899, p. 441.

[c] Loeffler and Frosch: Bericht der kommission zur erforschung der maul und klauenseuche bei dem Inst. f. Infek.-krank. in Berlin. Centbl. f. bakt. u. infek., 1898, bd. 23.

[d] Nocard: La "horse sickness" ou "maladie des chevaux de l'Afrique du Sud. Bull. de le soc. centr. de med. vet., n. s., vol. 19, 1901, p. 37.

[e] Journ. comp. path. and therap., 1900, XIII.

filtered through a Chamberland B filter for two hours under a pressure of 29 inches of mercury, the filtrate was infective to a horse into which it was injected. After an incubation of three days and a clinical course of six days the horse died.

Exudative typhus of chickens.—Magiora and Valenti[a] made experiments with the emulsions of the blood, lungs, liver, spleen, kidneys, and heart. They used the Berkefeld filter and Chamberland F. Dilutions were made with 40 and 60 parts of physiological salt solution and a pressure of 1½ atmospheres was employed.

Bacteriological examination of the filtrate proved negative, but chickens injected with 5 cc. of the filtrate died in about two days, presenting the same clinical and pathological picture as the naturally infected ones.

Another set of chickens injected with the blood of the ones which had received the filtrate developed typical symptoms and post-mortem changes of the disease.

An exposure of five minutes at 65° C. sterilizes the virus.

These investigators found that the filtrates had very much less virulence than the unaltered blood. Four cubic centimeters were found to represent the minimum fatal dose of a filtrate from a mixture of blood and water in the proportion of 1:160, whereas 4 cc. represented the minimum fatal dose of an unfiltered mixture of blood and water in the proportion of 1:1,500,000.

It is interesting to note how the filtration experiments cleared up an error which former students had made in regard to the loss of virulence in pure cultures of organisms which they had isolated in this disease. They had found a cocco bacillus in the internal organs. The culture tubes inoculated from the body fluids showed growths of this organism. Inoculations into chickens from colonies on the first set of cultures caused the disease, but subcultures from the first set of cultures were not virulent. Into the first set of cultures there had evidently been carried some of the invisible virus along with the cocco bacillus.

Mosaic disease of the tobacco leaf.—Beijerinck[b] pressed the sap out of diseased plants and passed it through very thick porcelain filters, and the filtrate was free from bacteria, but virulent for the tobacco leaf.

Epithelioma contagiosum of fowls.—It was found by Marx and Sticker[c] that the infective agent suspended in sodium chlorid solution passed through a Berkefeld filter, but not through a porcelain filter. The filtrate gave no growth on media; it was carried through a series

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[a] Magiora and Valenti: Ueber eine seuche von exsudativen typhus bei hühnern. Zeit. für hyg. und infekkr., vol. 42, 1903, p. 198.

[b] Centrbl. für bakt., Abt. 2, bd. 5, 1899, p. 27.

[c] Deut. med. wochenschr., bd. 28, 1902, p. 892.

of 16 generations in fowls; it resisted 60° C. for three hours, and after one hour in a vacuum tube at 100° C. it was virulent.

Hydrophobia.—Remlinger and Riffat-Bey [a] ground up a rabbit's brain in water together with a bouillon culture of chicken cholera and filtered it by aspiration through a Berkefeld V filter. The filtrate inoculated into rabbits caused rabies.

Celli and de Blasi [b] ground the brain and spinal cord in sand under 300 atmospheres pressure. A suspension in distilled water, when subjected to a small Berkefeld filter under a vaccuum of 570 mm. for half an hour, gave an infective filtrate.

Remlinger (Ann. de l'Institut Pasteur, v. 17, No. 12, 1903, p. 834) confirmed his earlier work with Riffat-Bey mentioned above. He showed that the virus of hydrophobia can not be made to pass through a Chamberland filter nor through a Berkefeld N or W. It can only be forced through a Berkefeld V, which filter is the most porous of the Berkefeld system.

Hog cholera.—De Schweinitz,[c] in a preliminary note, mentions a disease peculiar to hogs, indistinguishable clinically and at postmortem from hog cholera, but which can be transferred from hog to hog by inoculation with certain body fluids which have been rendered free from bacteria by filtration through the finest porcelain filters. This filtrate was shown to contain no organisms of hog cholera or swine plague, because when inoculated into rabbits and guinea pigs the animals remained healthy.

Rinderpest.—Nicolle and Adil-Bey [d] passed the virus of this disease through a Berkefeld filter, but not through a Chamberland F.

Clavelee (sheep pox).—Borrel [e] filtered a suspension of the pustules in water. The filtrate from the Berkefeld filter was infective, but that from the Chamberland F was not.

Nonfilterability of vaccine and smallpox.—Parke [f] crushed vaccine virus with fine sand, using 25 tons of pressure to the square inch. One portion of the suspension of crushed virus was passed through a Berkefeld filter and another portion through a Chamberland filter. Both filtrates were evaporated over sulphuric acid in a vacuum.

Calves and rabbits inoculated with the filtrate before and after

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[a] Remlinger and Riffat-Bey: Le virus rabique traverse la bougie Berkefeld. Compt. rend. heb. des Sec. de la Soc. de Biol., vol. 55, 1903, p. 730.

[b] Deut. med. wochenschr., vol. 29, p. 945.

[c] U. S. Dept. of Agriculture, Bur. Animal Industry, Circular No. 41, Sept., 1903.

[d] Nicolle et Adil-Bey: Études sur la peste bovine. Ann. de l'Inst. Pasteur, vol. 16, 1902, p. 56.

[e] Borrel: Experience sur la filtration du virus claveleux. Compt. rend., Soc. de Biol., vol. 54, 1902.

[f] Assn. Am. Physicians: Trans., vol. 17, 1902.

evaporation failed to show any reaction. Control animals inoculated with the crushed material before filtration always had successful vaccinations. The object of the crushing was to liberate the organisms from epithelial cells or other tissues which might retain them.

Smallpox virus from three fatal cases failed after crushing to pass into the filtrate, as determined by the inoculation of monkeys.

Filterable bacteria.—Von Esmarch [a] sought to determine whether there are such things as ultramicroscopic organisms among the saprophytes.

We readily believe that the virus of a filterable infectious disease is made of very small organisms, possibly ultramicroscopic, and that if these organisms could be made to multiply the resulting mass would have an appreciable size. If there are ultramicroscopic saprophytes he thought that all conditions were in the highest degree favorable for their multiplication, and that on the ordinary laboratory media they ought to find their most suitable conditions of growth and give an appreciable evidence of their existence.

He used 40 different kinds of fluids, including sewage, rich vegetable infusions, decomposing urine, emulsions of sputum, cavaders, and feces. The clear filtrates from these suspensions were planted on all the laboratory media and these plants kept under different conditions showed no growth.

During the first week's observations of the original filtrate no growth was noted; but after ten days this fluid showed a turbidity which was due to a very fine motile organism (*Spirillum parvum*), which grew as vibrios and spirilla, which were recognized only by the greatest magnification. It passed the Berkefeld, Chamberland F, Reischel, and Pukall filters and appeared in the first 200–300 cc. of filtrates. No other bacteria were found in the filtrates. Its size is about the same as that of the influenza bacillus, being 1 to 3 micra in length and 0.1 to 0.3 micra in width.

Von Esmarch grew bacteria through filters which hold them back in ordinary filtration work. He used Berkefeld, Kitasato, and Maassen filters.

These filters were filled with plain bouillon and were placed in a vessel containing bouillon inoculated with an organism, and the whole was kept at 37°, or room temperature.

Typhoid grew through the Kitasato filter at 37° in twenty-four hours, and at room temperature in two days.

Cholera went through a Maassen filter at 37° in two days, but a control kept at room temperature did not grow through after thirteen days.

A small Berkefeld filter allowed *Bacillus prodigiosus* to pass in

[a] Centbl. fur bakt., bd. 32, 1902, p. 561.

from one to three days, and a large Berkefeld allowed *pyocyaneus* and *prodigiosus* to pass in seven days.

Wherry [a] states that the bacillus producing pneumonia in guinea pigs (0.5 micron wide and 0.7 micron in length) passed the small Berkefeld No. 5, but was not found in the filtrate from the thicker walled Berkefeld No. 8, nor in the filtrate from the Chamberland F. It, however, grew through the walls of all three.

FOMITES.

While we made no experiments directly designed to determine the part played by fomites in transmitting the infection of yellow fever, still our work strongly bears on this point, and we can fully corroborate the conclusions of Reed and Carroll that fomites or inanimate objects are not dangerous in this respect.

Nonimmunes whom we kept for weeks under observation in our mosquito-proof rooms slept on the same beds, used the same clothing, washed from the same bowl, ate the same food, drank the same water, and breathed the same air as those sick with yellow fever; nevertheless they remained free of all fever except that which was purposely given them by mosquito bites or blood inoculations.

As these experiments were done in the summer time at Vera Cruz, a badly infected city where the disease prevailed at the time in epidemic form, it removes some of the objections which were made at the time to the work of the Army Commission, which for the most part was done during the winter months in an otherwise healthful locality—Camp Lazear.

THE FILTRATION OF MALARIAL BLOOD.

The filtration experiments with malaria were undertaken with the hope that they would throw light upon yellow fever, which bears so many analogies to malaria. Both diseases are transmitted by mosquitoes, and it is therefore natural to suppose that yellow fever is due to an animal parasite, perhaps similar to the well-known plasmodium of Laveran. However, as the one disease is filterable and the other is not; and as the parasite of the one is visible and the other can not be seen with the highest powers of the microscope at present at our command, either in the mosquito or in man; and as the one produces an immunity and the other does not, we find the analogy is not after all so very striking and that it does not seem helpful in solving our problem.

The malarial rosette breaks and liberates spores (*merozoites*) which are exceedingly minute, and in order to carry out the analogy in an

[a] Journ. med. research, vol. 8, 1902, p. 322.

experimental way we filtered malarial blood in order to determine whether there might be forms of the malarial parasite which are even smaller than this spore.

We know from the work of Novy that a trypanosome (*Trypanosoma Lewisi*), which is a colossal organism when compared with a malarial spore, has forms which are so minute that they pass a Berkefeld filter, for he has succeeded not only in artificially cultivating the adult trypanosome parasite, but in infecting animals with the filtrate from these cultures. We also know from the recent work of Schaudinn[a] that some of the animal parasites (*Spirochaeta*), multiply by reducing division; that is, each time cleavage takes place the organism is reduced in size, and this process continues until the divided forms become too small to be seen as individuals and can be made out only as clusters.

We therefore reasoned that if the malarial parasite has an ultramicroscopic form minute enough to pass the pores of a filter, it would encourage us very much to look for a visible form of the yellow fever organism in the blood and tissues of man and the mosquito by the aid of technique that had not previously been employed.

Our filtration experiments with malarial blood resulted negatively so far as demonstrating the presence of a minute or ultramicroscopic form of this parasite was concerned, but there developed unexpectedly what appears to be a demonstration of the malarial toxin. We produced a definite paroxysm by the inoculation of blood serum freed of the malarial parasites by filtration; and it is reasonable to suppose that the same substance circulating in the blood, which caused the chill, fever, and sweat in one man, caused a precisely similar chain of symptoms in the other two into whom this serum was transferred.

We found that if the blood is drawn after the height of the paroxysm and while the fever is declining this poison is not manifest; but if the blood is taken during the chill and while the temperature is rising, it is present.

If this poison is the toxin causing a malarial paroxysm it is remarkable that it should be present in the blood serum in such a considerable quantity and disappear so very rapidly. Still, the clinical symptoms of the disease would indicate the sudden production of a large quantity of toxin and its rapid elimination, neutralization, or destruction. So far as we know, this is the first time that a poison has been demonstrated which is capable of reproducing the symptoms of a disease due to an animal parasite of microscopic size.

It would be folly from a few observations to claim that we have discovered the malarial toxin. The only conclusion justified is that we have demonstrated the existence of some poison in the blood which is

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[a] Loc. cit.

capable of reproducing the symptoms of the disease when injected into the veins of other men.

We are not unmindful of the fact that chemical substances derived from the hemoglobulin or other proteids in the blood may be toxic, and we are of course familiar with the work of Gauldi, Montesano, Mannaberg, Celli, and others, who failed to demonstrate a pyrogenic toxin in malarial blood from similar experiments. The length of time the blood was exposed to the air between the time it was drawn from the malarial patient until it was injected into the person experimented upon may account for the discrepancies in results. The time the blood is drawn in relation to the paroxysm and many other factors should also be taken into account.

Mannaberg [a] drew blood during the attack in a case of ordinary tertian malaria. He centrifugalized it and injected the clear serum subcutaneously into two healthy people.

One received 1 cc. of serum at 4 p. m., when his temperature was 36.7° C. The temperature at 4.30 p. m. was 37° and at 6 o'clock 36°.

The other patient was given 0.7 cc. of the serum and his temperature rose within fifteen minutes after the injection from 36.5° to 37.6° C.

Celli [b] took during the cold stage a small quantity of blood from each of many malarial patients.

Young children were inoculated with 50 cc. of the serum subcutaneously and 50 cc. intravenously.

Another child was given the concentrated serum remaining after treating 260 cc. of serum in a vacuum apparatus at low temperature. The child was injected intravenously and subcutaneously.

From a hemorrhage in a case of severe comatose pernicious malaria 25 cc. of serum were obtained and injected into another patient.

None of the patients into whom the serum was injected showed pyrexia. There was in several instances, however, a slight rise of temperature which the experimenter says may occur after the injection of normal serum.

Rievel and Behrens [c] studied a sarcosporidium of the llama. They removed ten of the sacks and ground them up with physiological salt solution in a mortar, and injected 2 cc. of the fluid subcutaneously into a rabbit. After seven hours the rabbit died. The autopsy revealed nothing unusual.

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[a] Mannaberg, Julius: Die malaria krankheiten. Nothnagel's Specielle Pathologie und Therapie, Bd. 2, 1899.

[b] Celli, Angelo: Malaria. Transl. by J. J. Eyre. Longmans, Green & Co., New York and London, 1900.

[c] Rievel and Behrens: Beiträge zur Kenntnis der Sarcosporidien und deren Enzyme. Centralblatt für bakt. u. parasit. (orig.). Bd. 35, no. 3, s. 341.

Another rabbit received by mouth the contents of several sacks rubbed up in salt solution. This rabbit was given at the same time a subcutaneous injection of 1 cc. of the fluid. The animal died after eight hours. From a gross examination of his internal organs and a bacteriologic examination of the same, nothing abnormal was found.

Blood from the spleens of the above-mentioned rabbits, when injected into three other rabbits, caused no abnormal symptoms.

Pieces of the flesh of the llama were cut up in salt solution and the fluid part was injected into two rabbits subcutaneously. Both remained sound.

A rabbit inoculated subcutaneously with a suspension of the contents of sarcosporidia sacks in salt solution died after seven hours. The post-mortem was negative.

Two other rabbits treated in the same way remained alive six hours. Another died after seven hours.

A suspension was subjected to dialysis and it was found that the dialysat, when injected into a rabbit, caused death within twenty-four hours. The cooked dialysat was inactive.

Our experimental cases in malaria follow:

Filtration Experiments with Estivo-Autumnal Fever.

Filomena Martinez (case LXIII), 35 years old, born in Mexico City, lived in Vera Cruz about one year.

The patient was admitted to the hospital of Working Party No. 2 October 27, at 10 a. m. He had been under observation the previous day and early that same morning at San Sebastian Hospital.

As he showed a heavy infection with malarial parasites he was transferred to our laboratory.

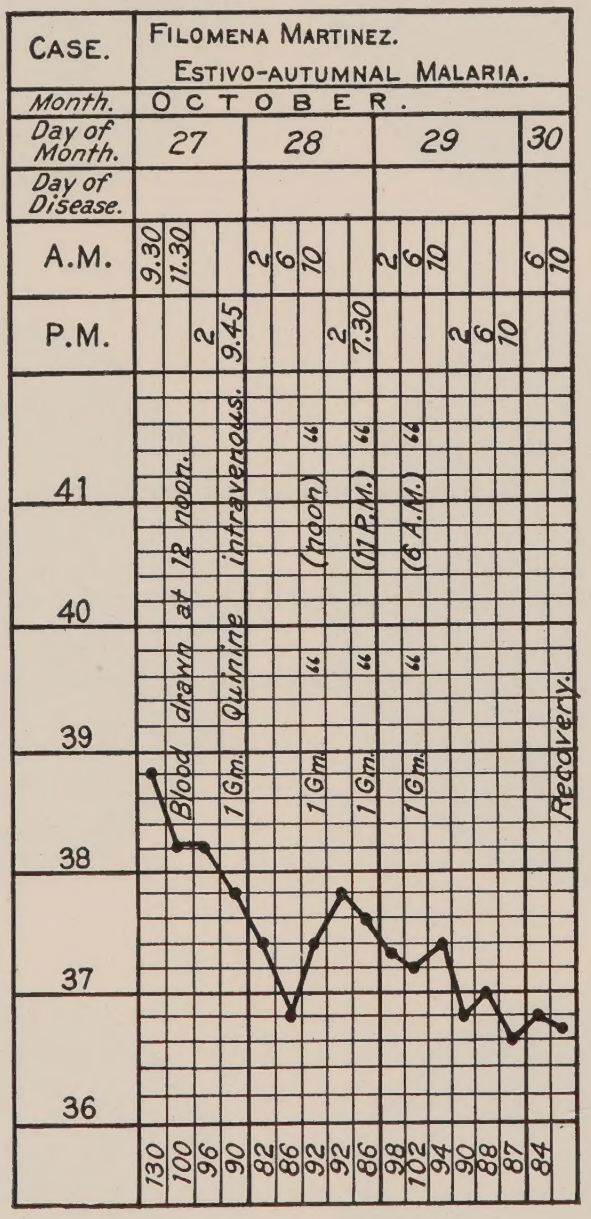
He gives a history of having had yellow fever about six months ago. His present illness, according to his statement, began some two weeks ago with fever, but he says he did not have chills. The patient's mental condition when seen was below par, and he was unable to give consistent answers. He seemed somnolent and was evidently beginning to show the effects of his infection upon the brain.

An examination of his blood, taken at 4.15 p. m., October 26, showed very many young ring forms, some of them with active amœboid shapes. None appeared pigmented in the smears stained with Goldhorn's polychrome methylene blue. Crescents and ovoids also present.

At 12 o'clock noon, October 27, a trifling incision was made through the skin over the median cephalic vein on the right side. A needle was introduced into the vein and 100 cc. of blood were quickly drawn into a sterile flask. The wound was covered with a sterile dressing and healed without complications.

The blood was immediately put into the ice chest, the temperature of which registered between 16° and 19° C. Clotting took place rapidly. The red cells settled to the bottom of the flask, the upper part of the clot being composed of a firm yellowish buffy coat. The serum separated well and was very clear.

The blood serum was drawn off and diluted with an equal volume of an isotonic salt solution and then divided into two portions.



Temperature chart of Filomena Martinez.

One portion was passed through a Chamberland B filter and injected into José Ojeira.

The other portion was passed through a Berkefeld filter and injected into Luis Peredo.

Blood smears made from the blood which was drawn from the vein showed in stained specimens crescents and the young small ring forms of estivo-autumnal malaria. Some of the stained parasites showed one chromatin point, others two. A few were irregular in outline,

EXPLANATION OF PLATE 2.

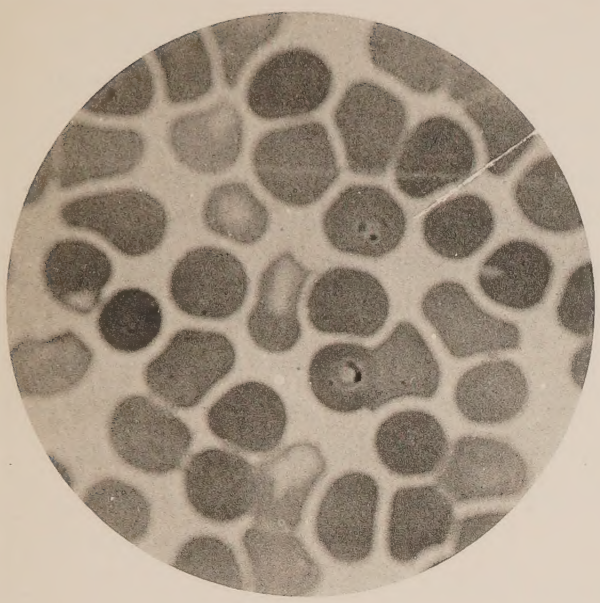
Estivo-autumnal malaria.

The character of the malarial parasites in the blood of Filomena Martinez the time it was filtered.

Stained with Goldhorn's polychrome methylene blue.

1. Small ring forms.
2. Young ameboid forms.

3, 4, 5, and 6. Ovoids.



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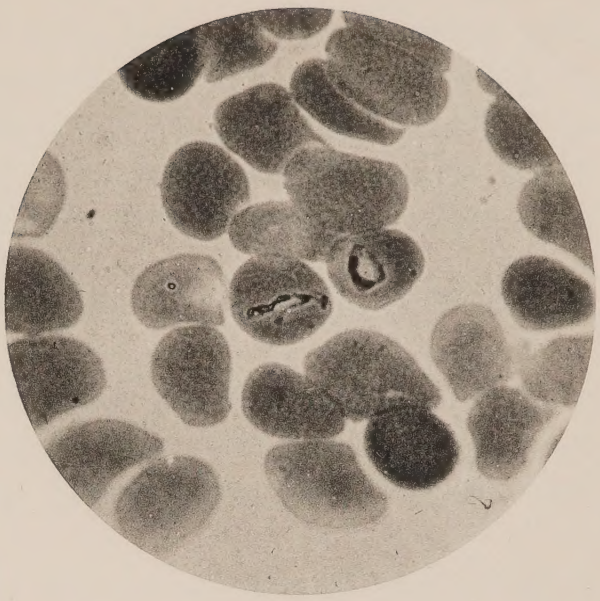

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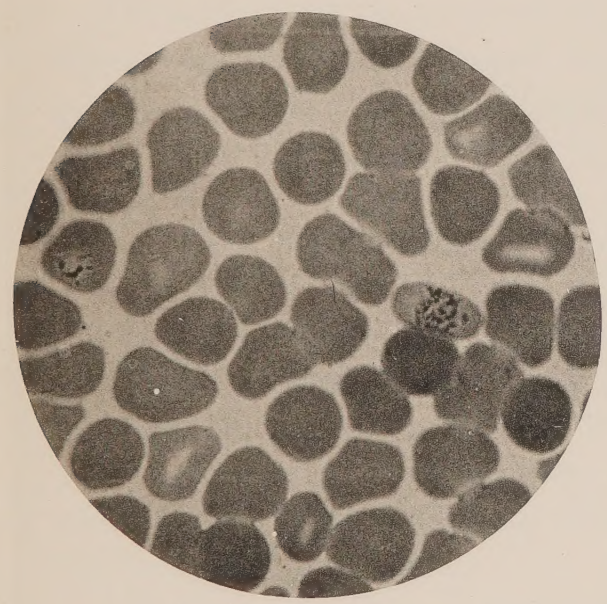

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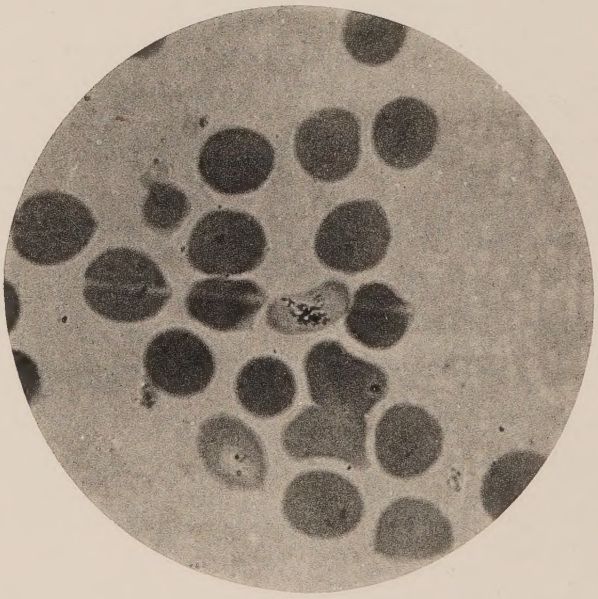

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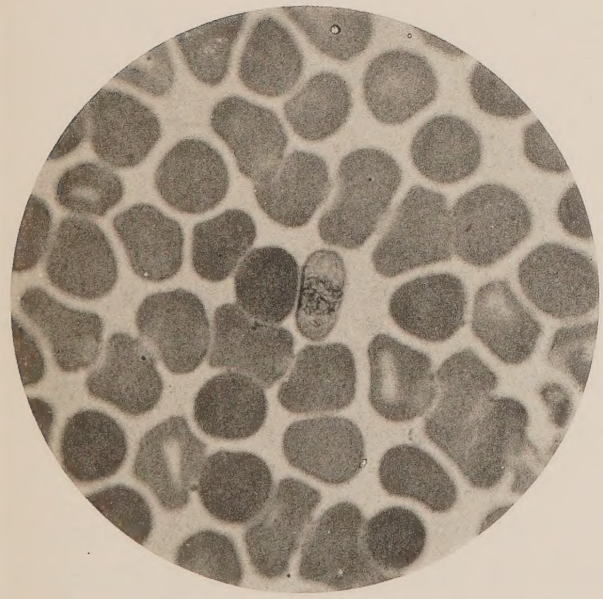

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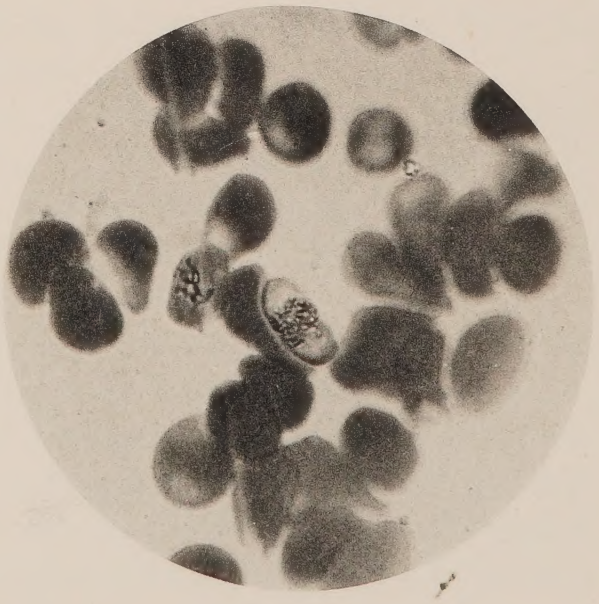

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CHARACTER OF THE MALARIAL PARASITES IN THE BLOOD OF FILOMENA MARTINEZ AT THE TIME IT WAS FILTERED.

indicating older parasites with amœboid motion. These latter were two or three times the size of the small ring forms. (See plate 2.)

These irregularly shaped parasites had two and some three chromatin points. In the blood taken at subsequent periods similar forms were seen, the older or younger forms predominating, depending upon the time of day the blood was examined.

The details of diluting and filtering the blood serum of Filomena Martinez follow:

At 5.30 p. m. the blood was taken from the ice chest, having been there just five and one-half hours, and 27 cc. of the clear serum were pipetted off. This serum contained a few flakes and very few red blood cells.

To this serum was added an equal amount (27 cc.) of physiological salt solution (0.6 per cent).

The mixture was transferred to a filter flask and a Chamberland B filter was carefully lowered into the fluid and securely fastened in position. This was a new filter marked as follows: "B. filtre Chamberland système Pasteur H. B. Cie., Choisy-le-Roi. BTE S. 6.O.G. Contrôlé."

The filter was tested before using with an air pressure of 30 pounds, after which it was lowered into water. When first lowered into the water the air came from every part of the surface of the filter in very fine bubbles, but nowhere was there evidence of a crack or pinhole. As soon as the filter became wet no air could be forced through it with a pressure of 30 pounds. This particular candle was considered to be tighter than the other Chamberland-Pasteur filters which we had similarly tested.

The filter was then thoroughly washed by allowing 200 cc. of water to pass through it under 20 pounds pressure. It was sterilized in the hot air sterilizer for one hour on the day before the blood was filtered, at a temperature of 150° C. for one hour.

The filtration was begun at 6 p. m., October 27, and was conducted in accordance with the diagram (fig. 2) by means of pressure from an air pump. This air pump was worked by hand and the diluted blood serum filtered under a pressure of 15 pounds. The pressure was controlled by the gauge, as shown in the sketch.

Very slight variations occurred both above and below 15 pounds, owing to the difficulty of exact control with hand power.

The pressure was kept up for one hour, and the filtrate was drawn from the inside of the bougie with a long sterilized pipette. In this manner it will be noticed that there was no possible chance of contact between the filtrate and the blood serum, and throughout the process the greatest care was taken in order to prevent such a contamination. The filtrate as it came through the filter was clear and of amber color.

At 7.20 p. m. 20 cc. of the filtrate were injected by means of an appropriate syringe with hypodermic needle into the left median cephalic vein of José Ojeira with entirely negative results.

A second portion of the blood serum of Filomena Martinez was

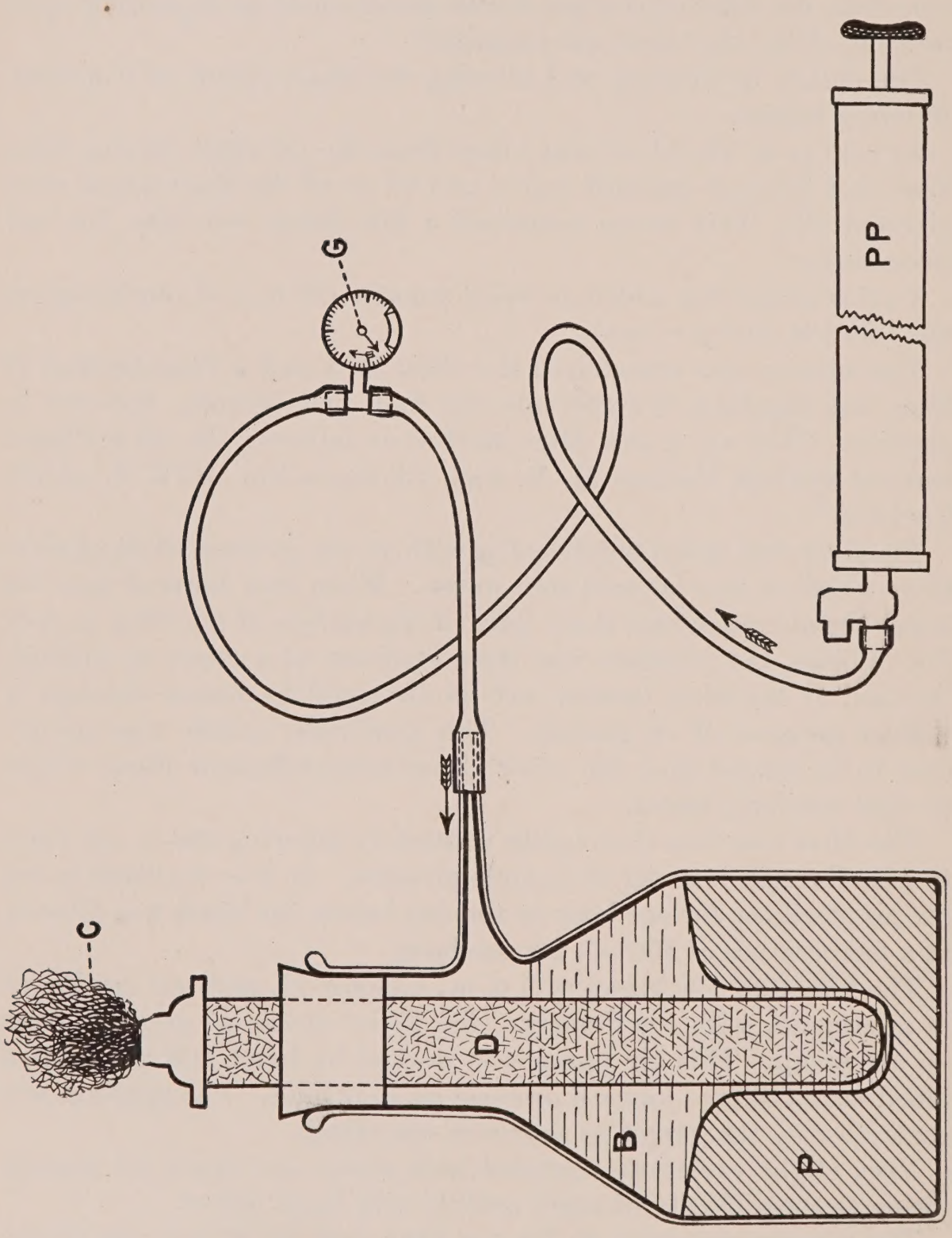


FIG. 2.—The arrangement used for filtering through a Pasteur-Chamberland bougie B. PP, pressure pump. G, pressure gauge. D, Pasteur-Chamberland bougie, stopped with cotton and sterilized; held in place by a rubber stopper. P, a molded cup of paraffin to keep the small quantity of blood in contact with the surface of the filter. B, the blood serum.

pipetted off and diluted with an equal quantity of physiological salt solution, and filtered through a small Berkefeld filter, as follows:

A new Berkefeld filter was prepared by thoroughly washing by allowing water to pass through it for several hours, and then sterilized in dry heat.

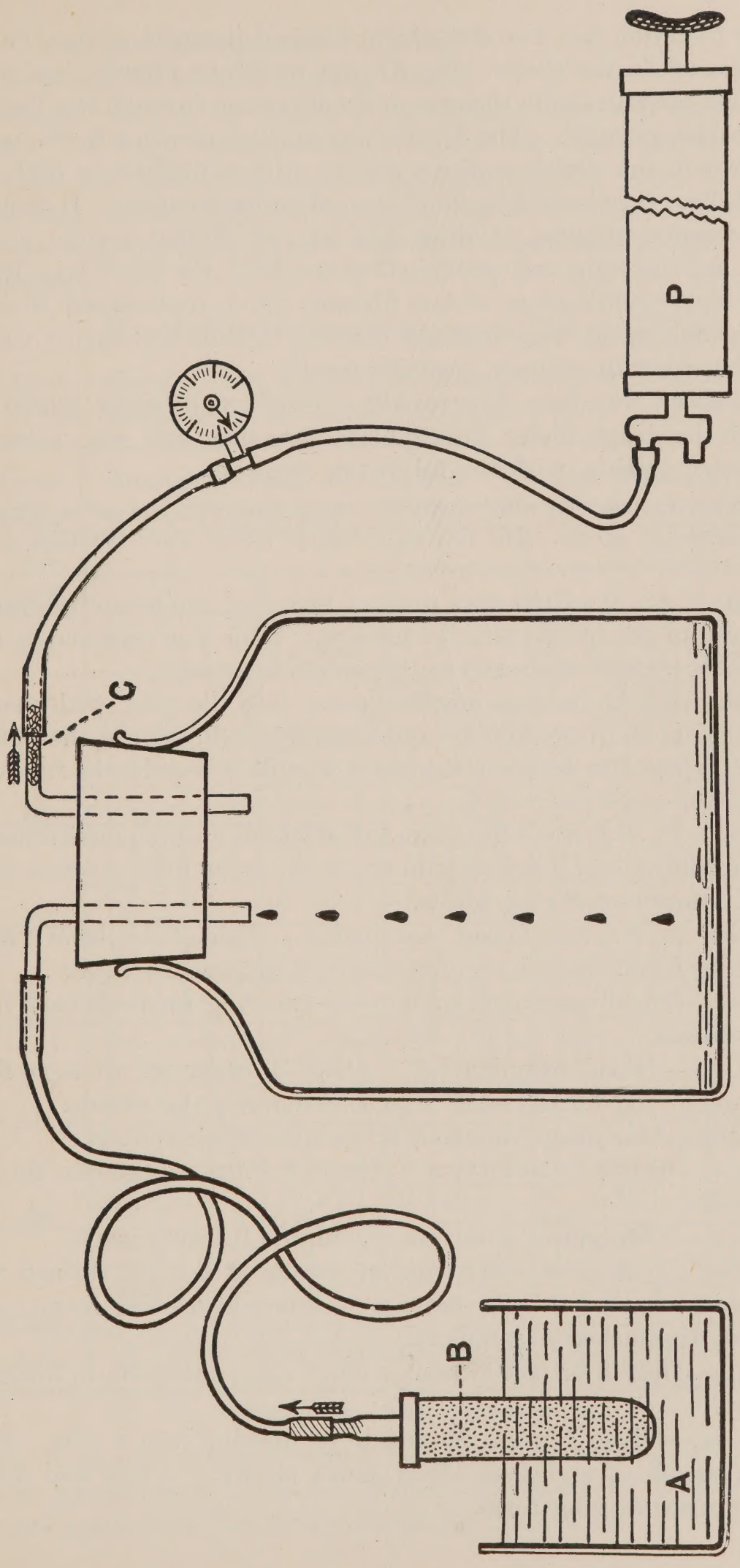


FIG. 3.—Showing the method of filtering through a Berkefeld filter by means of a hand vacuum pump. A, flask containing the blood serum. B, Berkefeld bougie. C, cotton plug. P, hand pump.

This filtration was also done from without inwards, as may be seen by reference to the sketch (fig. 3), but by using vacuum instead of direct air pressure, as in the case of the filtration through the Pasteur-Chamberland bougie. The filtrate was carried over to a sterile bottle, as shown in the sketch, and drawn out with a pipette, so that, care being taken, there could be no chance of contamination. It required about twenty minutes to filter 46.5 cc. of diluted serum, and at 8.15 p. m., viz, eight and one-fourth hours after the blood was drawn, twenty cubic centimeters of this filtrate, which represented 10 cc. of the original serum, were injected into the left median basilic vein of Luis Peredo with entirely negative results.

This filter was then thoroughly washed with water which ran through in drops under atmospheric pressure, and was preserved for further testing, with the following results:

On March 1, 1904, this filter was tested with a bouillon culture of *Staphylococcus pyogenes aureus*. The filtrate remained sterile after ten days in the incubator.

At 10 p. m. Martinez was given 1 gram of bimuriate of quinine directly into one of the veins of his arm. This was repeated at noon on October 28, and again at 11 p. m. on the same day.

On the 29th he received another gram into the vein, with marked improvement in his symptoms and a notable reduction in the number of intracorpuscular forms in the blood, as will be seen by the following notes of the case:

October 27, 10 p. m.—One gram bimuriate of quinine intravenously. Blood examination, 3 crescents in one field; some fields have as many as half a dozen small ring forms.

October 28, 2 a. m.—Blood examination. Some fields have 5 and 6 small ring forms, some irregular amœboid shapes, also ovoids.

6 a. m.—Blood examination. One or two ring forms to each field; also crescents.

10 a. m.—Blood examination. About 1 organism to each field; some young ring forms; some amœboid shapes; also ovoids.

12 noon.—One gram bimuriate of quinine intravenously.

2 p. m.—Blood examination. About 1 intracorpuscular form to each field.

11 p. m.—One gram bimuriate of quinine intravenously.

October 29, 2 a. m.—Ten-minute search of a slide stained with polychrome methylene blue showed no intracorpuscular forms; crescents not diminished in numbers.

6 a. m.—Only 2 intracorpuscular rings seen; crescents in moderate numbers.

10 a. m.—Given 1 gram bimuriate of quinine into a vein. Five-minute search of a stained blood smear shows 5 ovoids and only 1 intracorpuscular ring form.

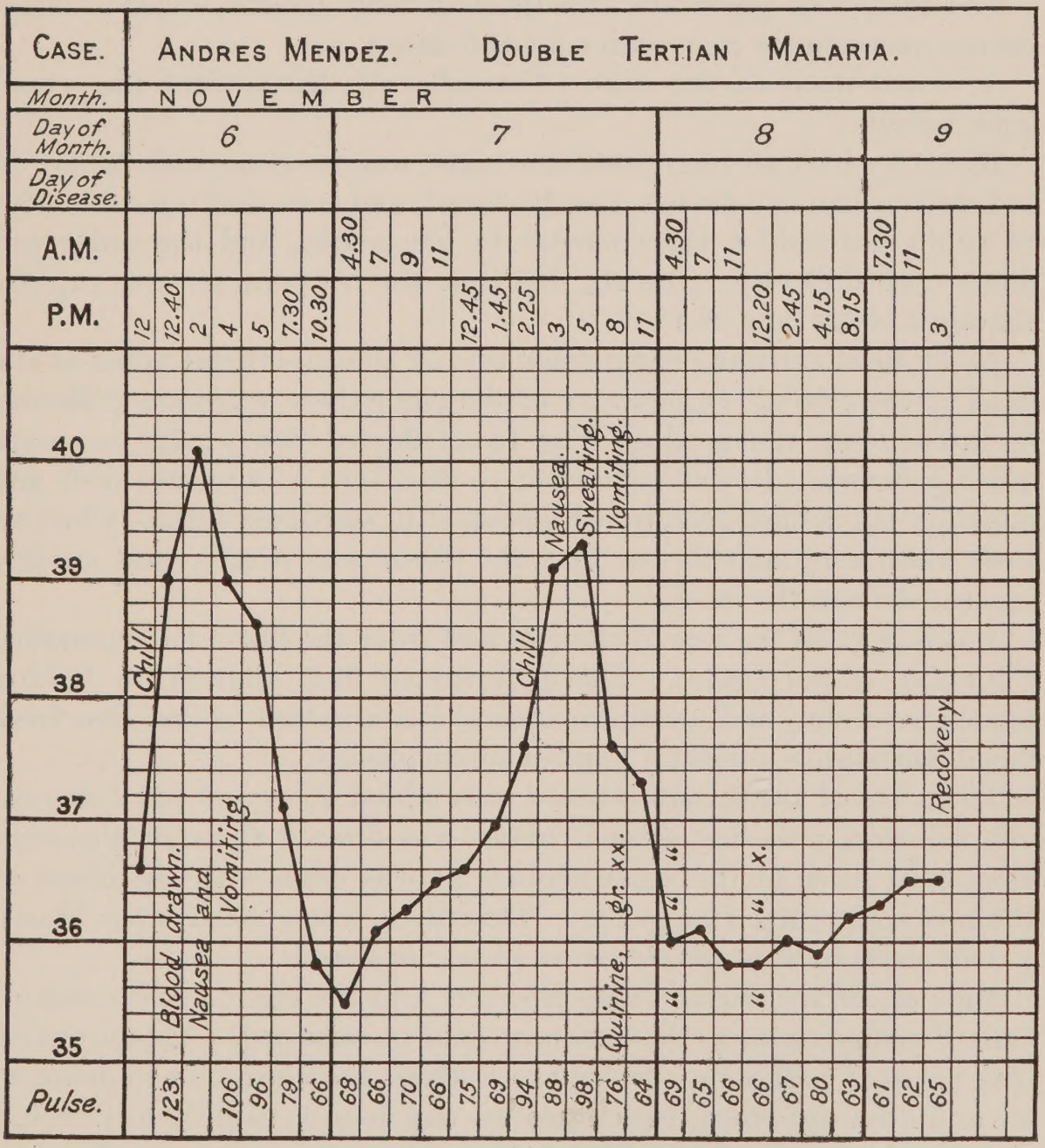
2 p. m.—No intracorpuscular forms.

10 p. m.—No intracorpuscular forms.

October 30, 6 a. m.—Blood examination shows no intracorpuscular forms. A large mononuclear leucocyte has much brown pigment. Ovoids still present in apparently undiminished numbers. Patient has very much improved in his general condition. His mind is better, appetite has returned, and he was returned to San Sebastian Hospital.

Filtration Experiments with Tertian Fever.

Andrez Mendez (case LXVI), 39 years old; born in La Luz, Estado de Guanajuato; never had fever in his native place. In 1878 had yel-



Temperature chart of Andres Mendez.

low fever (?) in San Antonio, Estado de Guanajuato, with which he says he was sick about one month. He came to Vera Cruz three years ago and has had fevers five or six times since.

Present illness dates from about November 3, but states that he has been troubled with mild attacks of fever for a month, which he describes as coming on alternate days, but not sufficiently severe to keep him from his work.

The fever which initiated his present sickness began with a severe chill, and was followed by fever and sweat, and was associated with some nausea and vomiting. He states that these paroxysms were repeated daily until his admission to San Sebastian Hospital, November 6, 1903.

Blood examination showed that he had a heavy infection with tertian parasites, and he was immediately transferred to the laboratory of Working Party No. 2, Yellow Fever Institute.

The man was physically robust, but very anemic, mucous membranes particularly pale, skin cold and damp.

At about noon on this date (November 6), the patient was seized with a chill.

By 12.30, half an hour later, the rigor was very marked; he lay in bed with a blanket drawn over his head, and was shaking violently; he could not hold a thermometer in his mouth, and the pulse was taken with difficulty. During this time the temperature was rapidly rising, it being now 39.1° C.

At 12.40 blood was drawn from one of the superficial veins at the bend of the elbow. On account of the rigor there was some difficulty in introducing the needle. The blood flowed freely; 125 cc. were quickly drawn. It was permitted to flow into a porcelain dish and immediately defibrinated by whipping with sterilized forks. Clotting took place very quickly, so that the fibrin was readily and quickly separated from the fluid.

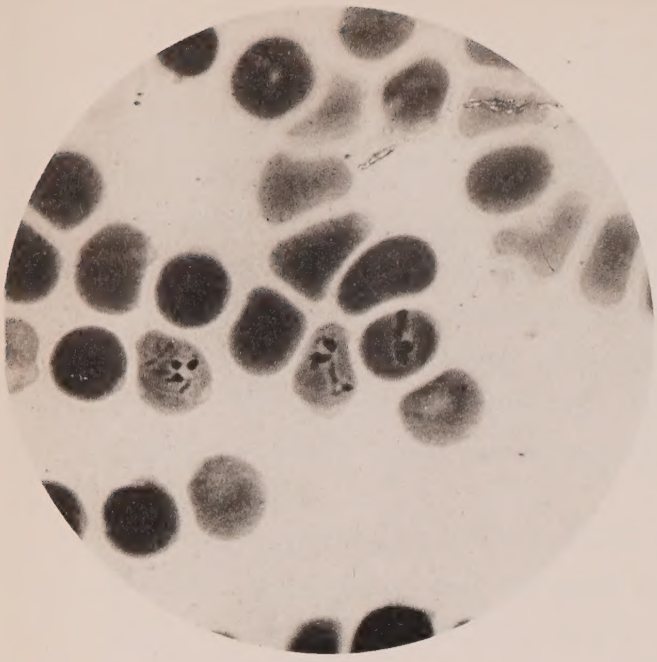
Judging from the size of the clot and color the fibrin had enmeshed a number of corpuscles. The defibrinated fluid showed no further tendency to clot, and on microscopical examination looked like fresh blood containing a normal number of corpuscles.

To 25 cc. of defibrinated blood was added 25 cc. of physiological salt solution, and this diluted blood was filtered through the same Berkefeld filter in the same manner as was done with the blood of Filomena Martinez (see p. 84). This filter, when tested later, March 1, 1904, held back *Staphylococcus pyogenes aureus*.

Nine cc. of the filtrate were injected into the right basilic vein of Luis Peredo as soon as this amount could be obtained. This injection took place at 1.40 p. m. It only took about forty minutes to defibrinate and filter the blood, which process was done as rapidly as possible.

Stained smears of the filtrate showed no morphologic elements. The filtrate had a distinct red color. For the method by which this filtration was done, see fig. 3.

As a control, José Ojeira, at 2 p. m., was given an injection into



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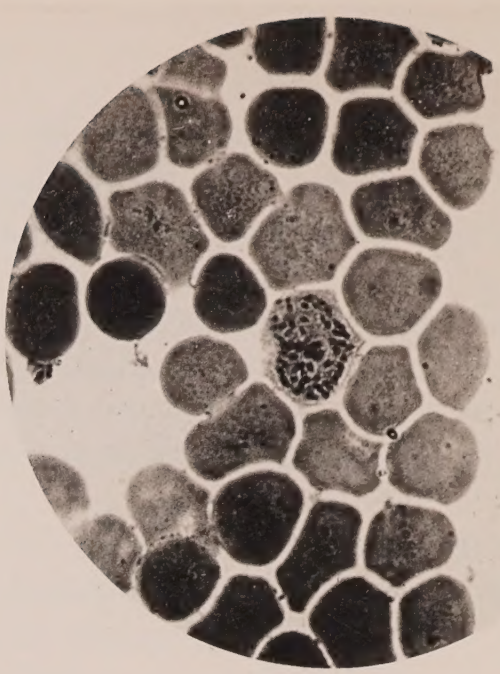

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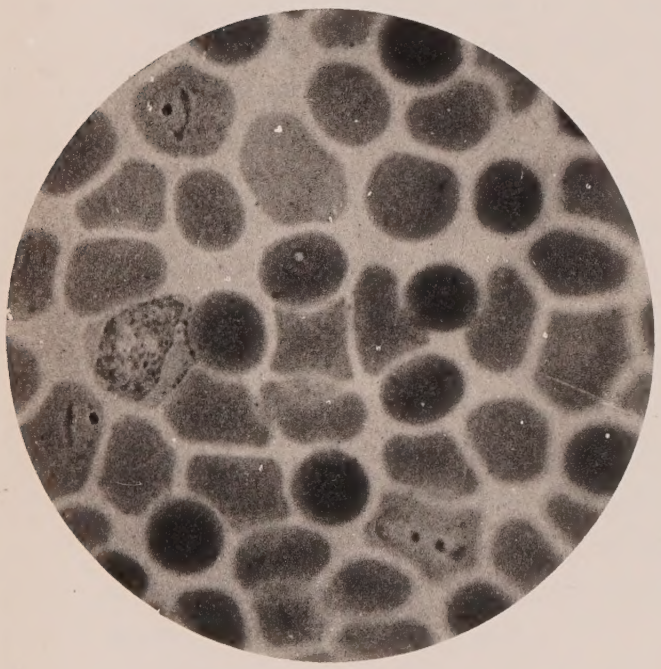

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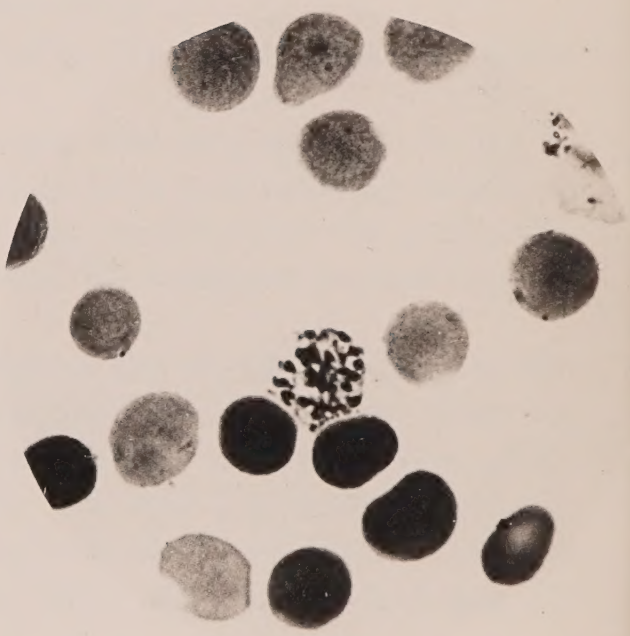

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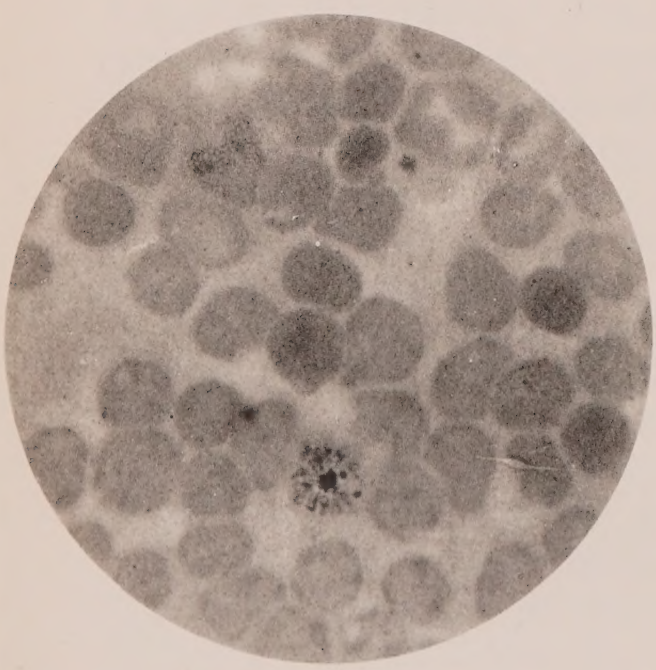

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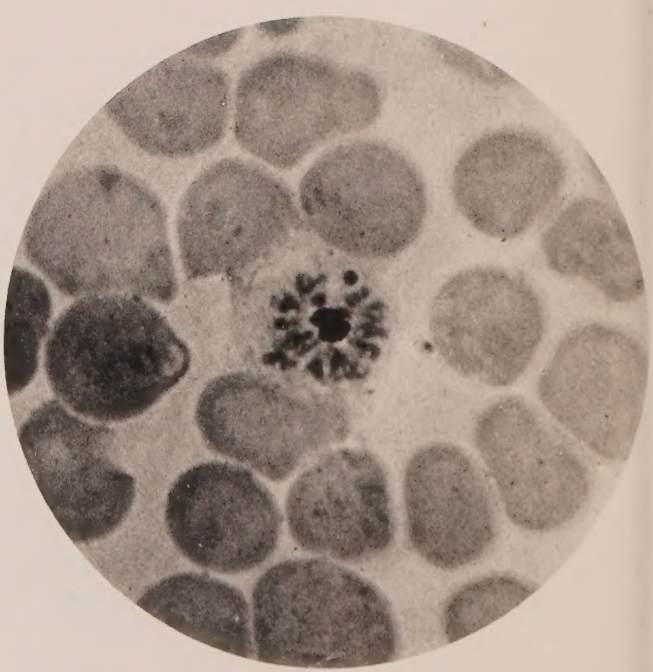


6

CHARACTER OF THE MALARIAL PARASITES IN THE BLOOD OF ANDRES MENDES AT THE TIME IT WAS FILTERED.

EXPLANATION OF PLATE 3.

Double tertian malaria.

The character of the malarial parasites in the blood of Andres Mendez at the time it was filtered.

Stained with Goldhorn's polychrome methylene blue.

1. Young ameboid forms.
2. Older pigmented parasite.
3. Young and old forms in the same field.
4, 5, and 6. Segmenting forms.

his left basilic vein of 4 cc. of the *unfiltered* mixture. As the blood was diluted with equal parts of salt solution, he therefore received 2 cc. of Mendez's blood.

The unfiltered mixture of defibrinated blood and salt solution, upon microscopic examination shortly after Ojeira received his injection, showed amoeboid tertain organisms with dancing pigment. For the character of the malarial parasites infecting the blood of Mendez, see illustration, plate 3.

After drawing the blood from Mendez he continued to have a chill, with severe rigor and chattering of the teeth, accompanied by nausea and vomiting. His temperature continued to rise after the blood was drawn until it reached 40.2° C. The febrile period was followed by drowsiness and moisture of the skin.

As will be seen by reference to the temperature chart, Mendez was kept under observation without quinine, and had another typical malarial paroxysm the next day. All the evidence in his peripheral blood, which was examined frequently, pointed to a severe double infection with the tertian parasite.

He was then given quinine, which entirely controlled the disease, and caused the complete disappearance of the parasites from his peripheral blood.

The results caused by the injection of the blood of Andres Mendez into Peredo and Ojeira follow:

Luis Peredo (case LXIV), a volunteer, aged 25, born in Jalapa, State of Vera Cruz, where he has always lived. When examined at Jalapa, August 26, he was found to be physically sound; urine contained no albumin; peripheral blood showed no plasmodium.

He was brought to Vera Cruz August 28 and taken from the station directly to the laboratory, from which time he was kept constantly within a mosquito-proof room.

On October 27, after having been under daily observation two months, during which time he remained in normal health, he was injected with the filtered blood of Filomena Martinez (page 84), who at the time was suffering with a paroxysm of malarial fever of the estivo-autumnal type, his blood containing many young ring-forms and crescents.

It will be noted by reference to the records of Filomena Martinez that the blood was drawn during the decline of the paroxysm. It was then allowed to clot in the ice chest; the clear serum was pipetted off and diluted with an equal quantity of isotonic salt solution, and this filtered through a new Berkefeld filter.

Twenty cubic centimeters of the filtrate, which on account of the dilution represented 10 cc. of the blood serum, were injected into the left median basilic vein of Peredo.

For further details of the manner in which the blood serum was obtained and the filtration performed, see the above records of Filomena Martinez.

Peredo was carefully watched from the hour he was injected, but he remained in good health, and no deviation from the normal was detected.

His temperature was taken every four hours during the night and day, both before and following the injection, as will be seen by the temperature chart. No symptoms developed.

His blood was examined daily for plasmodium, but none was found. The result of this injection must therefore be considered negative.

Ten days later he was again injected with filtered malarial blood under different circumstances, and with positive results.

At 1.40 p. m., November 6, he was given an intravenous injection of the blood of Andres Mendez, passed through the same Berkefeld filter as before. Mendez was suffering with a double tertian infection; his blood was drawn during his chill and before the height of the paroxysm, as will be seen by reference to the temperature chart (page 85).

Thinking that allowing the blood to clot four or five hours in the ice chest in order to obtain a clear serum for filtration might be too severe a tax upon the vitality of the malarial parasite, we this time defibrinated the blood as quickly as possible, diluted it as before with an equal volume of physiological salt solution, and filtered it through the same Berkefeld filter in the same manner as was done with blood of Filomena Martinez.

As soon as 9 cc. of the filtrate could be obtained it was injected into the basilic vein of the right arm of Louis Peredo. This injection took place at 1.40 p. m.

About thirty-five minutes after receiving the injection he began having chilly sensations and headaches, and presently went to bed covering himself with his blanket (2.25 p. m.). Five minutes later (2.30) he was having a violent chill, his teeth chattering so that we could not trust the thermometer in his mouth. The rigor of the entire body was so marked that there was difficulty in taking the radial pulse. The face was pale, and at this time he vomited most of the dinner he had eaten a short time before receiving the injection.

The patient complained of headache, which he localized at the forehead and occiput; says he felt cold and had pains in the knees. At this time the skin was dry. The chill lasted somewhat over half an hour.

At 3 p. m. the patient had transient chilly creeps, very slight rigor.

At 3.15 p. m. he said he felt " warm inside," and all sense of chilliness had disappeared; still has headache.

At 3.25 p. m. he complained of marked pain in the legs.

At 3.30 p. m. vomited the remainder of his dinner.

It will be seen from the temperature chart that during this time his temperature was rapidly rising and reached its highest point (38.7° C.) at 4 o'clock p. m., just two hours and twenty minutes after receiving the injection.

The pains in the knees and back continued, and nausea and vomiting now became a distressing feature of the paroxysms for the patient.

The fever gradually subsided, and reached normal at 4.30 a. m. that same night. (See temperature chart.)

As the fever subsided the skin became moist, the nausea and pains gradually disappeared, so that by 6 o'clock p. m. the patient was quiet and dozing. The entire paroxysm, therefore, according to the temperature record, lasted about eight hours, although the patient was sleeping quietly five hours after receiving the injection.

It is interesting to note that this man Peredo had what seemed to be a typical malarial paroxysm beginning with a distinct rigor associated with a rise of temperature and followed by slight sweating. It is of particular interest to note that his paroxysm, so far as symptoms were concerned, was very much like the paroxysm from which Andres Mendez suffered, especially the nausea and vomiting.

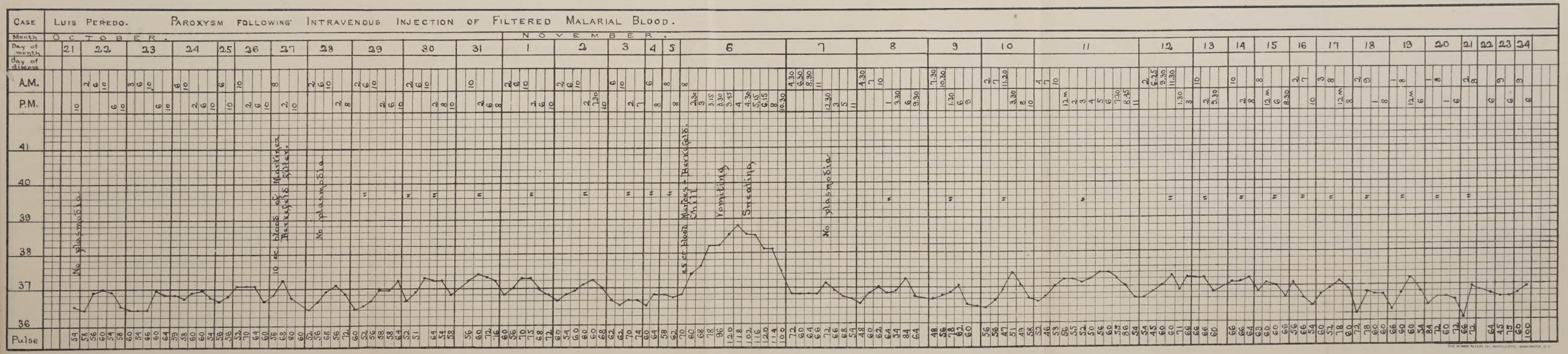
Peredo was kept under very close scrutiny until November 24, eighteen days following the injection, during which time he remained entirely normal and no plasmodium appeared in his peripheral blood, which was frequently examined, as follows:

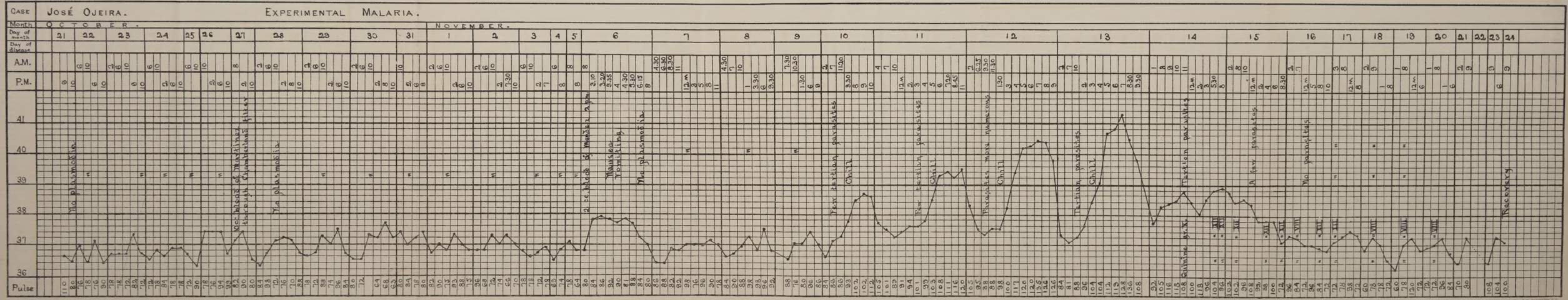
Blood examination.—Goldhorn's stain.

November 6.—4.30 p. m., 8 p. m. No malaria.
November 7.—4.30 a. m., 8.30 a. m., 12.30 p. m., 5 p. m., 11 p. m. No malaria.
November 8.—7 a. m., 1 p. m., 6 p. m., 9.30 p. m. No malaria.
November 9.—7.30 a. m., 1.30 p. m. No malaria.
November 10.—2 a. m., 3.30 p. m., 8. p. m., five minutes each. No malaria.
November 11.—4, 7, 10 a. m., 2, 6, 11 p. m., five minutes each. No malaria.
November 12.—1.30, 6.25 a. m,, five minutes each. No malaria.
November 13.—7 a. m., 9.30 p. m., five minutes each. No malaria.
November 14.—8 a. m., 8 p. m., five minutes each. No malaria.
November 15.—8 a. m., 8.30 p. m., five minutes each. No malaria.
November 16.—7 a. m., 9.30 p. m., five minutes each. No malaria.
November 17.—8 a. m., 8 p. m., five minutes each. No malaria.

José Ojeira (case XXIII), a volunteer from Jalapa, 18 years old; had never lived on the coast, and says he never had fever of any kind. On examination in Jalapa, August 11, he was found to be physically sound, of robust physique; urine showed no albumin, and blood examination for malaria was negative.

He was taken to Vera Cruz August 13, and immediately transferred to a mosquito-proof room in the laboratory, where he was kept under close observation.


Case
Luis Peredo.
Paroxysm following Intravenous Injection of Filtered Malarial Blood.
October.
November.
Day of month
Day of disease
A.M.
P.M.
Pulse
No plasmodia.
10 cc. blood of Mariquez Bacteriolic Chart.
No plasmodia.
0.5 cc. blood Mariquez + Bacteriolic.
Chill
Vomiting
Sweating
No plasmodia.


Case
José Ojeira.
Experimental Malaria.
Month
October.
November.
Day of month
Day of disease
A.M.
P.M.
Pulse
No plasmodia.
Few hyaline & pigmented hæmatozoa.
No plasmodia.
Few hyaline pigmented hæmatozoa.
No plasmodia.
Few hyaline pigmented hæmatozoa.
No plasmodia.
Few hyaline

On August 28, at 9.30 a. m., he was bitten by four mosquitoes, two of which had bitten Antonio Leal (case XXXV), a yellow-fever patient fifteen days seventeen hours previously; and the other two had bitten the same case fourteen days twenty-three hours previously.

The man was kept under close observation in a mosquito-proof room, but showed no reaction. There was no rise of temperature, nor did he present any untoward symptoms.

On October 27, 7.20 p. m., he received intravenously 20 cc. of diluted blood serum of Filomena Martinez (æstivo-autumnal infection), passed through a Pasteur-Chamberland filter B. This represented 10 cc. of blood serum. For detals of this filtration see Filomena Martinez (p. 81).

Ojeira showed no reaction whatever as a result of this injection.

It will be noted that the blood of Martinez was drawn after the height of the paroxysm, and while the temperature was on the decline. Martinez was suffering with a very severe æstivo-autumnal infection at the time the blood was taken.

Ojeira's blood was carefully examined several times daily, both before and following this experiment, and at no time was anything resembling a malarial parasite seen in his peripheral blood.

On November 6, the patient having continued in good health since the last experiment, was used as a control for the experiment made on Peredo.

On this date, at 2 p. m., he was given an intravenous injection of 4 cc. of the *unfiltered*, diluted, and defibrinated blood of Andres Mendez. At the time the blood was drawn from Mendez it contained a heavy infection of a double tertian malaria, and the blood was taken from him during a chill and before the height of his paroxysm. It was at once defibrinated, diluted with an equal volume of physiological salt solution, and filtered through a Berkefeld filter. Nine cc. of the filtrate were given intravenously to Peredo, causing a malarial paroxysm without, however, the presence of the malarial parasite, and due, as we believe, to the toxin (?) in the blood of Mendez.

Ojeira, who received 2 cc. of unfiltered blood (4 cc. dilution), reacted within an hour, with a slight rise of temperature and nausea, and four days following developed a typical malarial paroxysm, with many tertian parasites in his peripheral blood.

There can be no doubt that the reaction to the 2 cc. of defibrinated blood injected into the vein of Ojeira caused a slight paroxysm, which it is reasonable to suppose was due to the same poison present in the blood of Mendez, and which also caused the reaction in Peredo.

It will be noticed that 2 cc. of this blood caused but a slight reaction in the case of Ojeira, while 4.5 cc. caused a more marked reaction, with a rise of temperature to 38.7° C., in the case of Peredo,

indicating in a very definite manner that the severity of the symptoms were directly due to the quantity of poison introduced. Ojeira did not have a chill or other manifestations of a malarial paroxysm, other than a rise of temperature and nausea. He vomited gastric mucus several times.

On November 10, the fourth day following the injection, Ojeira had a typical malarial paroxysm, with tertian parasites in his peripheral blood. He suffered with a double infection, having a chill every day, as will be noticed by reference to the temperature chart.

The character of the parasites in his blood and the clinical course of the disease resembled in all respects those of Mendez, from whom the blood was taken. Both cases were entirely controlled by quinine.

MISCELLANEOUS OBSERVATIONS ON MOSQUITOES.

In association with Doctor Goldberger we made some miscellaneous observations upon the life history and biology of the *Stegomyia fasciata* and *Culex pipiens*, some of which were of sufficient interest to record.

We found that the female *Stegomyia* does not always lay her eggs at one time. More often she deposits them in groups at intervals of several days. The maximum number laid by any one insect observed by us was 101. The female *Stegomyia* sometimes dies, apparently from exhaustion, after laying her eggs, but we noted several instances in which this was not the case. The insect which laid 101 eggs was alive and vigorous until killed by us five days later.

The manner in which the female *Stegomyia* lays her eggs is of some interest. She bends her abdomen ventrally, and as the genital orifice comes in contact with the sides of the vessel or with the water or other object the egg is deposited. The insect moves along and repeats the performance.

The eggs are sometimes laid on the water, sometimes on the side of the vessel above the water line, and sometimes on a leaf floating on the water.

In accordance with the few observations which we made on this particular point, *Stegomyia fasciata* females that are fed solely on banana or sugar do not lay eggs. They seem to require a feeding of blood for ovipositing. Unconjugated females also do not lay eggs.

The longest life that we observed was in a female *Stegomyia*, which we kept for sixty-four days, and then killed for sectioning.

We found, as has been often noted by other observers, that the greatest activity of the *Stegomyia fasciata* is during the daytime, but we have observed them flying at night. We have also observed them feeding on us at night by artificial light. We have noticed on several occasions that they are especially voracious early in the morn-

ing, about sunrise. On several occasions a number of noninfected insects were let loose in the laboratory and we observed that upon rising at sunrise they attacked us viciously.

It may be noted that this fact apparently explains the danger to persons sleeping in an infected house and the comparative freedom from danger in daylight communication with an infected town, especially if the person remains in the open air and sunlight and avoids houses, and confines his visits to the streets and parts of the town free from the disease.

A number of experiments were made with the female *Culex pungens*, but they could not, under any circumstances, be induced to feed upon blood while in confinement. It was found that they preferred death.

MOSQUITOES MAY BITE CADAVERS.

The female *Stegomyia fasciata* will bite a cadaver, and, if on a dependent portion, can draw blood. We have two observations on this point.

Narciso Nadal (case XX). A number of *Stegomyiæ* were applied twelve hours after death, only one of which apparently obtained blood.

Trinidad Martinez (case XXII). A number of female *Stegomyiæ fasciatæ* were applied one-half hour after death, and three insects succeeded in feeding with blood.

As it has been shown by the work of the French commission that the blood of yellow fever is not infective after the third day, the danger of conveying the infection by means of mosquitoes feeding upon cadavers must be exceedingly remote.

Longevity.

Several experiments were undertaken to determine the fact whether the male *Stegomyia fasciata*, as has been stated, has a brief life history. We have one experiment showing that the male mosquito may live and thrive over a month.

Observation.—A number of male mosquitoes were placed in a cage October 10 and subsequently fed on sirup. They were all alive and in good condition on November 15, when they were killed and used for experimental purposes, having lived thirty-six days.

Ovipositing.

Sometimes the female *Stegomyia fasciata* will lay a considerable number of eggs at one time and then die.

Observation.—(Mosquito XLIII. Francis, Re. No. 4.) This female *Stegomyia fasciata* was taken from the breeding jar on October 3, fed on normal blood October 4, 6, 7, 8, and 10, then placed in a jar with water to tempt ovipositing, and banana feeding begun. On October 19 four males were added to the jar. On October 23 the female was given another blood feed. On the 26th

she laid 54 eggs and was found exhausted and dying on the surface of the water.

More often the female *Stegomyia* lays groups of eggs at intervals of several days, and sometimes lives after the last laying.

Observations.—(Mosquito XLIII–38. Francis, Rc. No. 1.) This female *Stegomyia* was taken from the breeding jar October 3; fed on normal blood on October 4, 6, 7, 8, and 10; then placed in a jar to tempt ovipositing and given banana; laid eggs on October 10 and 11; given another blood feed October 16; found apparently dying on the surface of the water on October 18.

(Mosquito XLIII–68. Francis, Rc. No. 3.) This female *Stegomyia* was taken from the breeding jar on October 3 and fed on normal blood October 4, 6, 7, 8, and 10. It was then placed in a jar to tempt ovipositing and given banana. On October 11 she laid a considerable number of eggs. On October 19 again given blood feed and three males placed in the jar. October 25 she laid 29 eggs. On October 26 laid 12 more eggs. On the 30th the insect was removed in a dying condition.

(Mosquito XLIII–67. Francis, Rc. No. 5.) This female *Stegomyia* was taken from the breeding jar October 3. Fed on normal blood October 4, 6, 7, 8, and 10. Then placed in a separate jar with water to tempt ovipositing and given banana. On October 13 she laid some eggs. On the 19th given a blood feed and three males added. She laid 11 more eggs on October 23, 16 on the 25th, and 37 on the 28th, when she was found dying on the surface of the water.

(Mosquito XLIII–69. Francis, Rc.) This female *Stegomyia* was taken from the breeding jar when less than forty-eight hours old and fed upon normal blood October 4, 6, 7, 8, and 10, and subsequently at intervals of one or two days up to October 23, when she was placed in a cage by herself with a beaker of water to tempt ovipositing. At the same time given banana to feed upon. October 25 two males were added to the cage. October 25 she laid 51 eggs; November 7, 24 eggs; November 8, laid 26 eggs; total, 101 eggs.

She was given another feed of blood November 10.

Was killed November 13 while apparently vigorous.

Female *Stegomyia fasciata* that have fed on the blood of yellow-fever patients on the second, fourth, and sixth days of the disease may subsequently lay eggs that hatch in a normal manner, and the larvæ develop into pupæ and imagoes.

Observations.—(Mosquito XLVII–36. Marcial Lujan, Rx. a.) This insect was separated from the breeding jar October 8 and fed on Marcial Lujan, a typical case of yellow fever on the sixth day of his illness. She was subsequently fed on normal blood October 11, 13, 16, 17, and 19. On October 22 a beaker of water was placed in the cage in order to tempt ovipositing, and banana feeding begun. She laid 15 eggs on October 25 and 15 more on October 26. Both sets hatched.

(Mosquito XLVII–37. Marcial Lujan, Rx. b.) This female *Stegomyia* taken from the breeding jar October 8. Fed on blood of Marcial Lujan on the sixth day of his illness, October 9, and subsequently on normal blood October 11, 13, 16, 17, and 19. On October 22 given a beaker of water to tempt ovipositing and banana feeding begun. She laid 31 eggs on October 26, 9 on the 27th, and 12 on the 30th. The eggs laid on the 26th and 27th hatched on the 29th.

(Mosquito XLII–122. Marcos Cruz, Rn. a.) This insect was separated from the breeding jar October 14 and allowed to feed on Marcos Cruz, one of our experimental cases of yellow fever October 15, that is, on the second day of his

illness. She was subsequently fed on sirup. On November 1, placed in a cage with a beaker of water and two males. She laid 12 eggs on November 4, which subsequently hatched.

(Mosquito LVIII–32. Sesoleda Martinez, Rx. b.) This female *Stegomyia* was separated from the breeding jar October 19. Fed upon the blood of Sesoleda Martinez, a fatal case of yellow fever October 20, the fourth day of his illness. Subsequently this insect was given banana. October 28 a normal blood feed. On November 5 a beaker of water was placed in the cage to tempt ovipositing. Four days later, November 9, she laid 26 eggs, which subsequently hatched.

The statement has been made that the female *Stegomyia fasciata*, and mosquitoes generally, require a feeding on blood in order to lay eggs. In three experiments tried by us we are able to confirm this statement so far as the *Stegomyia fasciata* is concerned. The insects were fed on sirup and banana, but could not be tempted to lay eggs.

Observations.—Banana feeding. A large number of male and female *Stegomyia fasciata* that had been fed on banana for fourteen days were given a beaker of water to tempt ovipositing. They were left nine days. No eggs laid. They were then killed for section.

Sirup feeding. A large number of male and female *Stegomyiæ* that had been fed on sirup for fourteen days were given a beaker of water to tempt ovipositing. They were observed twenty-one days later. No eggs were laid.

Banana and sirup feeding. A large number of male and female *Stegomyiæ* were given alternate feedings of banana and sirup for thirty-two days, at which time a beaker of water was placed in their cage to tempt ovipositing. They were observed nine days later. No eggs were laid.

Unconjugated females do not lay eggs.

Observations.—Stegomyiæ pupæ were isolated and placed in separate small bottles so that the imagoes could not be kept in strict quarantine. Six of these unconjugated females were given a feeding of blood twenty-four hours after birth, and were subsequently fed on banana. They were kept in a cage with a beaker of water to tempt ovipositing. Five days subsequently they were given a second feeding on blood. Twenty-five days later they were killed, not having laid eggs.

Size of Screening.

It is of considerable practical importance in quarantine and public health work to know the size of screening that will keep out the *Stegomyia fasciata*, and as no accurate observations upon this subject had been made, with which we were familiar, we conducted a few experiments to determine this point.

Screens with a varying number of meshes to the inch were placed over breeding jars, and banana, sirup, and other food placed on the other side so as to tempt the hungry insects to pass through. These experiments were arranged by placing the fruit and food in a jar which was inverted over the breeding jar. A piece of gauze or netting was inserted between the two jars so that the *Stegomyiæ* would have to pass through its meshes in order to apear in the upper jar.

We found that both male and female *Stegomyiæ* may pass a wire

gauze containing 16 strands or 15 meshes to the inch, but could not pass 20 strands or 19 meshes to the inch.

It is therefore evident that the large-meshed mosquito bars ordi-

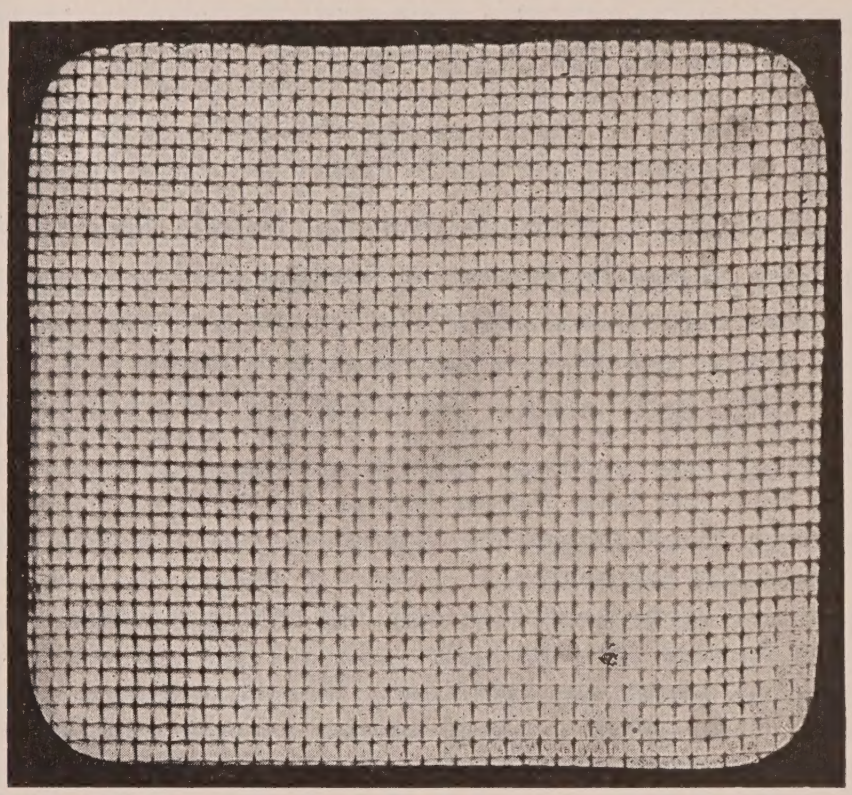


FIG. 4.—Showing screen containing 16 strands or 15 meshes to the inch. Allows male and female stegomyia fasciata to pass.

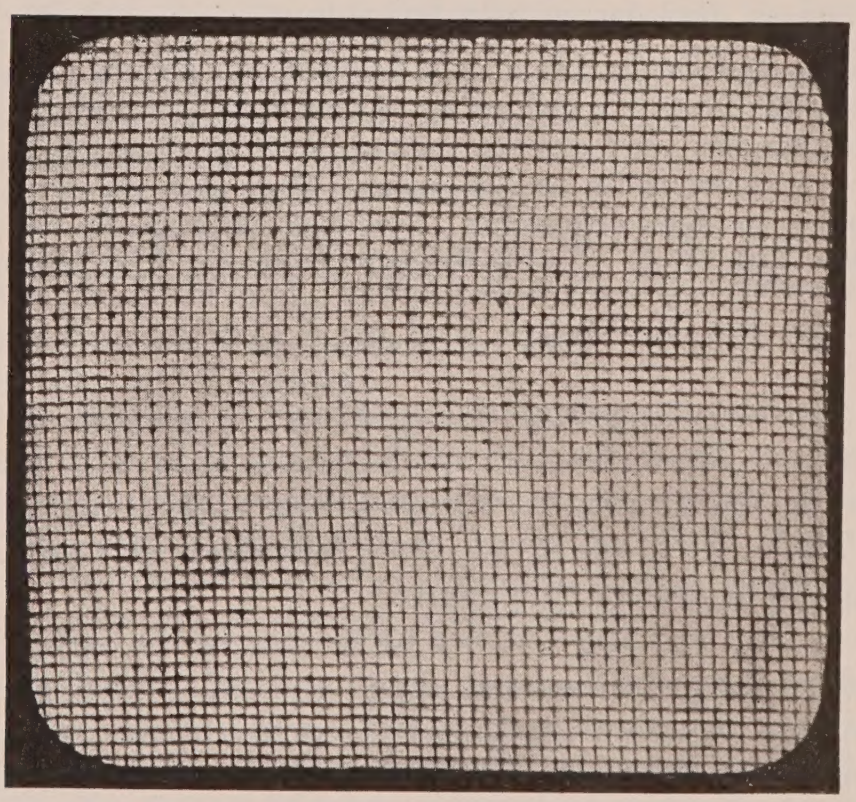


FIG. 5.—Showing screen containing 20 strands or 19 meshes to the inch, through which stegomyia fasciata can not pass.

narily used in this latitude would not offer proper protection, and that window screening must also be of a finer wire than is sometimes employed.

DISINFECTION EXPERIMENTS.

A few tests were made to determine the relative efficiency of sulphur dioxid, tobacco smoke, and pyrethrum as insecticides with particular reference to the *Stegomyia fasciata*.

A small room and hallway containing 1,200 cubic feet of air space were used in these experiments. The room contained a large window and one door, which were made reasonably tight to prevent the escape of the fumes, and the mosquitoes were exposed in cages in various parts of the room.

Experiment No. 1—Tobacco.—One pound of tobacco per 1,000 cubic feet; exposure, one hour; result, some mosquitoes survived.

Six hundred and twenty-five grams of tobacco, which is about the proportion of 1 pound per 1,000 cubic feet, were burned in a pan in the center of the room. The cages containing the mosquitoes were placed on the floor, near the ceiling, and on a chair.

The room was opened one hour after the tobacco was lighted, which was done by means of alcohol. The mosquito cages were immediately removed and placed in a current of fresh air in order to give the mosquitoes a favorable opportunity to revive.

All the mosquitoes in the cage which was near the ceiling were dead. Of those in the cage on the chair, one female was flying actively about; the other females and another male were all dead. Those in the cage that stood on the floor were stupefied, but none was killed, most of them flying actively about.

The tobacco was completely consumed in this process, and the fumes upon opening the door were very strong. The unpleasant odor was very persistent and disagreeable.

Experiment No. 2—Tobacco.—Two pounds per 1,000 cubic feet; exposure, two hours; result, all mosquitoes killed.

The mosquito cages were immediately removed after two hours had elapsed, at which time none of the insects showed apparent signs of life; but after remaining in the fresh air for three hours a few of them moved their wings and tarsi. None, however, revived.

Experiment No. 3—Pyrethrum.—Two pounds per 1,000 cubic feet; exposure, 2 hours; result, all mosquitoes killed.

The pyrethrum was burned in a brazier placed upon some sand on the floor.

Three cages containing many mosquitoes were distributed, one on the floor, one near the ceiling, and one in an open box on the table. The cages had a piece of crumpled gauze upon the bottom and a folded handkerchief hanging in the cage, in order to give the insects retreats in which to hide from the effects of the fumes and to test the penetrating action of the gas.

All of the mosquitoes were killed.

Experiment No. 4—Pyrethrum.—Two pounds per 1,000 cubic feet; exposure, two hours; result, all mosquitoes killed.

Mosquito cages were placed in several parts of the room. At the end of the experiment some of the insects showed signs of life, but none revived.

Experiment No. 5—Pyrethrum.—One pound per 1,000 cubic feet; exposure, two hours; result, all mosquitoes killed.

The pyrethrum was burned in an open brazier upon the floor in the same room used for the preceding experiments. The mosquitoes were freely exposed to the effects of the fumes in appropriate cages, which were placed upon the floor, near the ceiling, and one inside a box laid upon a table.

At the end of the experiment the cages were immediately taken to the fresh air and many of the *Stegomyia* showed signs of life, but none revived.

Experiment No. 6—Sulphur.—Three and one-fourth pounds per 1,000 cubic feet; exposure, two hours; result, all mosquitoes killed.

In this experiment four large rooms and the hallway of our laboratory building were fumigated to destroy some mosquitoes that had escaped. The rooms communicated with each other and with the hallway through large openings. No attempt was made to seal the doors and windows or to paste cracks.

Three pots of sulphur were distributed at points of vantage, each pot containing 20 pounds, the total space to be fumigated being 12,280 cubic feet.

Two hours after the sulphur was lighted the house was opened and it was found that only about two-thirds of the sulphur had burned; that is, about $3\frac{1}{4}$ pounds per 1,000 cubic feet. In this experiment the pots containing the sulphur were not placed in water, the object being to obtain a dry gas and thereby minimize the destructive action of the fumes upon the fabrics and pigments.

Four cages containing mosquitoes were placed in different parts of these rooms and all were killed.

As a result of these disinfection experiments we conclude that: While we found tobacco smoke efficacious in destroying the insects, we also found that the method is exceedingly objectionable on account of the persistent and disagreeable odor that it leaves, as well as on account of the yellow stains which remain. Tobacco smoke in concentrated form stains fabrics, paint work, and other surfaces, but the stains may be removed by washing; still, this forbids its use in parlors and the rooms of fine houses.

We found that the burning of 1 pound of tobacco per 1,000 cubic feet, with an exposure of one hour, was only sufficient to stupefy the insects. All *Stegomyia fasciata* were killed by an exposure of two

hours to the fumes of tobacco burned in a proportion of 2 pounds per 1,000 cubic feet.

We found 1 pound of pyrethrum per 1,000 cubic feet, with an exposure of two hours, sufficient to kill *Stegomyia fasciata*, and, although many of them still showed signs of life after this exposure, none revived. Two pounds per 1,000 cubic feet, with an exposure of two hours, was sufficient to kill them outright.

We made only one experiment with sulphur dioxid in order to kill some *Stegomyia fasciata*, which had escaped in the laboratory. We selected sulphur on account of our confidence in the insecticidal value of this substance. In this experiment we used about 3 per cent of the gas, generated by burning in open pots, with an exposure of two hours. The previous work of one of us[a] showed that 1 per cent, with an exposure of one hour, is quite sufficient in a small inclosure, but we used the excess on account of the peculiar construction of the house.

When burning sulphur for its insecticidal effects care should be taken to keep the gas as dry as possible, while the contrary is necessary in order to obtain its germicidal action.

The small percentages of sulphur dioxid kept dry are not sufficient during short exposures to bleach pigments or injure fabrics; and this, in our opinion, is the best of the poisonous gases to insure the destruction of such vermin in houses. Formaldehyde gas is utterly untrustworthy in this connection. It is not an insecticide.

We made no experiments with hydrocyanic-acid gas. While recognizing its great insecticidal power, we considered it entirely too poisonous a substance to use in the household or other inhabited places. In our opinion the use of this gas should be limited to greenhouses, railroad cars, granaries, and other isolated and uninhabited places.

SUMMARY AND CONCLUSIONS.

The cause of yellow fever is not known.

The *Myxococcidium stegomyiae* is not an animal parasite. Yeast cells sometimes simulate the coccidia in form and staining reaction.

The infection of yellow fever is in the blood serum early in the disease. No abnormal elements that bear a causal relation to the disease can be detected in the serum or in the corpuscles with the best lenses at our command.

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[a] Disinfection against mosquitoes with formaldehyde and sulphur dioxid, M. J. Rosenau. Bull. No. 6, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., Sept. 1901.

The infective principle of yellow fever may pass the pores of a Pasteur-Chamberland B filter.

Particles of carbon visible with Zeiss lenses pass through both the Berkefeld and Pasteur-Chamberland B filters.

Because the virus of an infectious disease passes a Berkefeld or Pasteur-Chamberland B filter it does not necessarily follow that the parasite which passed the filter is " ultramicroscopic," or that it may not have elsewhere another phase in its life cycle of large size.

The filtration of viruses may succeed or fail, depending upon the character of the filter, the diluting fluid, the pressure, time, temperature, motility of the particles, and other factors.

The period of incubation of yellow fever caused by the bites of infected mosquitoes is usually three days, sometimes five days, and in one authentic instance six days and two hours; but when the disease is transmitted by such artificial means as the inoculation of blood or blood serum the period of incubation shows less regularity.

Yellow fever may be conveyed to a nonimmune by the bite of an infected *Stegomyia fasciata;* but the bites of *Stegomyia* which have previously (over twelve days) bitten cases of yellow fever do not always convey the disease.

Fomites play no part in the transmission of the disease.

The tertian and estivo-autumnal malarial parasites will not pass the pores of a Berkefeld filter.

We have demonstrated a poison in the blood during the chill of tertian infection which, when injected into another man, caused chill, fever, and sweating. This poison, while present in a case of tertian during the rise of temperature, could not be demonstrated in the blood of a case of estivo-autumnal fever during the decline of the paroxysm. While this poison reproduced the symptoms of the disease, still the data are too limited to consider it the malarial toxin.

Stegomyia fasciata is a domestic insect. It is most active during the day, but will bite at night under artificial light. The female lays eggs at intervals; the maximum number of eggs laid by one insect observed by us was 101. The mosquito does not always die directly after ovipositing.

Stegomyia fasciata may bite and draw blood from cadavers, although the danger from spreading the infection from this source is remote.

Male and female *Stegomyia fasciata* may pass a screen containing 16 strands, or 15 meshes to the inch, but not one of 20 strands, or 19 meshes to the inch.

Tobacco smoke produced by burning two pounds per 1,000 cubic feet with an exposure of two hours is sufficient to kill *Stegomyia*

fasciata. This method is objectionable on account of the yellow stains and disagreeable odor.

Pyrethrum burned in the proportion of 1 pound per 1,000 cubic feet with an exposure of two hours will stupefy *Stegomyia fasciata;* it requires 2 pounds to kill them outright.

From the limited number of experiments made and from previous experiments we consider sulphur dioxid the best of the gaseous insecticides for this purpose.

Formaldehyd gas is not an insecticide, and therefore not applicable.

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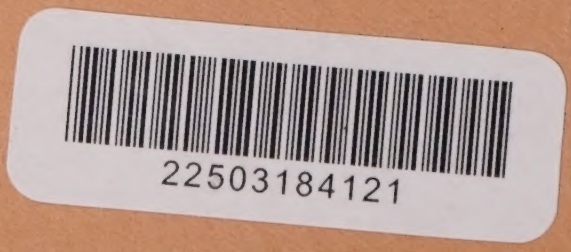
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